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1. Martin, G. A., Garfinkel, B. T., Brooke, M. M., Weinstein, P. P., and Frye, W. W.: *J.A.M.A.*, 151:1055, Mar. 28, 1953.
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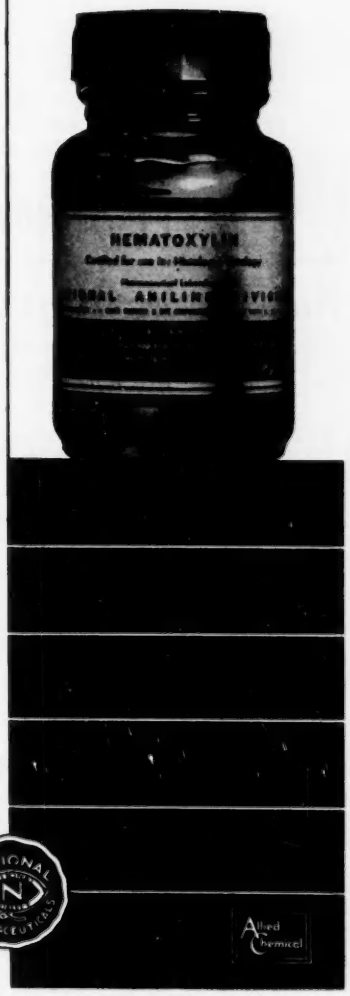

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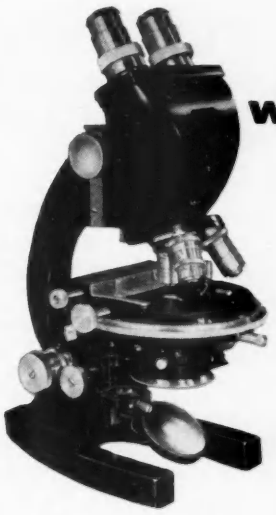

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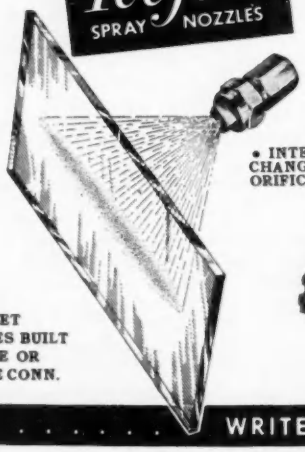

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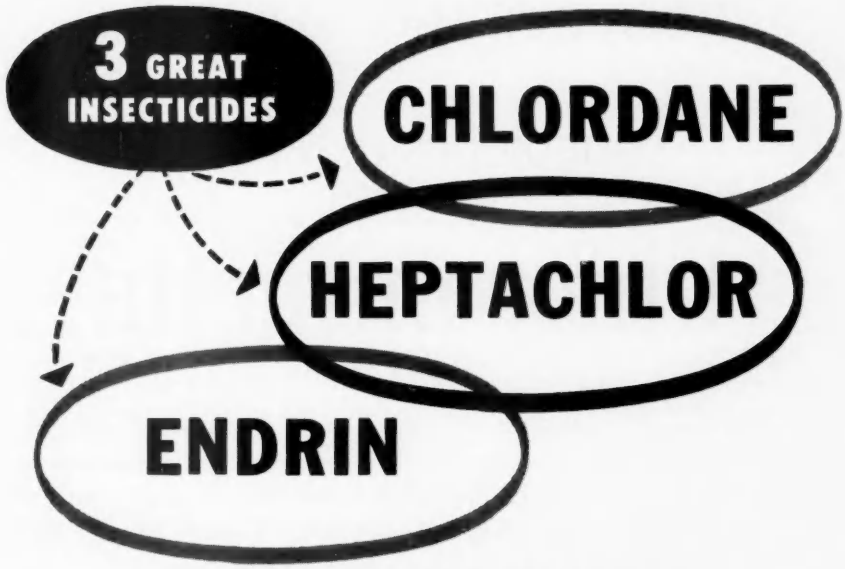

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(1) Kirby, W. M. M.; Waddington, W. S., & Doornink, G. M.: Antibiotics Annual, 1953-1954, New York, Medical Encyclopedia, Inc., 1953, p. 285. (2) Finland, M., & Haight, T. H.: *Arch. Int. Med.* 91: 143, 1953.

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FIFTY YEARS OF AMERICAN MEDICINE ON THE ISTHMUS OF PANAMA[1]

RICHARD MINTON, SIGFRID MULLER, AND GENE COHEN

In May, 1904, the United States obtained a strip of land 10 miles wide and extending 50 miles in length north and south across the Isthmus of Panama where construction was soon to begin on the epoch-making Panama Canal. During the past fifty years the Government of the United States has maintained a staff of doctors, nurses and medical technologists for the care of canal employees, merchant seamen, personnel of the Armed Forces and travelers at this crossroad of the Western Hemisphere. The importance of the medical department in the construction of the canal has often been reviewed, but many other accomplishments little known to the average person have also come from this area. Hundreds of interns and recently scores of resident physicians have received their training at Gorgas Hospital, and numerous physicians have visited the Canal Zone to see and study tropical diseases. Several important discoveries and many types of therapy originated here, and trials of new therapeutic agents have been conducted from time to time. Research in tropical medicine has been carried on continuously in the Canal Zone hospitals and laboratories since 1904, and also at Gorgas Memorial Laboratory in Panama City after its founding by an Act of Congress in 1928. An attempt will be made to review briefly these accomplishments on this occasion of the golden anniversary of the Panama Canal.

EARLY PERIOD: 1904–1920

The outstanding personality of this era was William Crawford Gorgas whose efforts in sanitation made possible the construction of the canal by the United States where others had only speculated, or tried and failed. In April, 1904, he was appointed Sanitary Officer of the Isthmian Canal Commission (ICC) and together with John W. Ross, U.S.N. and Louis A. LaGarde, U.S.A., made a tour of Panama to appraise the situation. He then went back to procure supplies and personnel for the mission. When he again arrived in Panama on the "Alliança" in June 1904, he was accompanied by the following people who comprised the first Medical Department: J. W. Ross, Medical Director; Henry R. Carter, USPHS, Chief Quarantine Officer; L. A. LaGarde, Superintendent, Ancon Hospital; Theodore Lyster, USA; J. C. Perry, USPHS; C. C. Pierce, USPHS; and also LePrince, Balch, Turtle and Miss Hibbard. One week later A. B. Herrick, E. P. Beverly, A. I. Kendall and Lloyd Nolan arrived from New York.

They found a situation which was far from assuring. There were only two cities, one at either end of the proposed canal: Panama City, with a population of

[1] Address request for reprints to Mrs. Virginia Stitch, Medical Librarian, Gorgas Hospital, Ancon, Panama Canal Zone, to whom the authors make grateful acknowledgment for invaluable help in the preparation of this paper.

24,500 on the Pacific (south) side, and Colon, with 2,000 inhabitants on the Atlantic (north) side. The Canal Zone was completely covered by a dense tropical forest watered by a monthly average of 10 inches of rainfall during the 8-month rainy season. Yet there was no safe water-supply for the residents. During the rainy season water was collected from roofs in barrels or cisterns, contaminated by the buzzards which constantly roosted on the house-tops. Swamps surrounded the townsites, and indeed the city of Colon was actually situated in a low partially filled swamp. There was no sewer system except for some antiquated underground stone drains that seeped sewage into the surrounding soil. The odor of Panama was recognizable two or three miles out at sea.

The hospital system taken over from the French was badly run down from lack of use. The hospital at Ancon, *l'Hôpital Central*, was maintained by the Sisters of a French religious order and was a picturesque structure situated on Ancon Hill overlooking Panama City. It consisted of fifty buildings having a capacity for 700 patients. The beautiful tropical shrubbery adorning the grounds and wards was the pride of the Sisters. Many of these plants were potted and were watered daily, so that in them and in the water-filled cans in which the legs of all the hospital beds rested to keep away the ants, ideal breeding sites were provided for mosquitoes. Indeed, mosquito larvae were found throughout the hospital, including of course those of *Aëdes aegypti* within the wards themselves, as well as various species of Aëdes, culicines, and anophelines in the pools on the grounds outside. In addition to the Ancon Hospital, there were Santo Tomás Hospital in Panama City and two small hospitals in Colon which needed complete reconstruction.

The death rates in the cities were high, and malaria, tuberculosis, beri-beri, dysentery and yellow fever were quoted by Gorgas as the leading causes of death in the order named. Yellow fever was low because of the stabilized immune population.

Gorgas organized the Health Department in four general categories: one to rid the area as far as possible of breeding places for mosquitoes; another to kill the adult mosquitoes; another to treat the sick and practice prophylactic measures; and lastly one to clean up the cities, pave the streets and supply a water and sewage system. His organization was given sweeping authority in the Republic of Panama, a unique concession for a sovereign state to make. These regulations exist to-day, although in practice most of the responsibility has been assumed by the Republic of Panama. Gorgas assumed charge of all that related to health and sanitation in the terminal cities with power to make and enforce by fine any regulation he deemed necessary. Under his jurisdiction United States physicians staffed Canal Zone hospitals and Santo Tomás, and treated all illness in the Canal Zone and Republic of Panama, including mental disease, leprosy and tuberculosis. The Public Health Service inspected and quarantined all vessels coming in contact with the terminal cities. The Sanitary Department inspected and fumigated all habitations in both cities, cleaning houses and yards that failed to meet its requirements. All wells and cisterns, pots and earthen jars were properly covered or destroyed. Disposal of garbage and night soil was

performed by the Health Department. Piping was laid from Gatun Lake to filtration plants and a complete and safe water supply system went into operation in 1914. A sewage system was also installed providing most inhabitants with inside toilets. The Sanitary Department cremated the dead and even employed its own clergymen to conduct the funeral services.

In 1905 the I.C.C officially established the Board of Health Laboratory in Ancon for the purpose of providing a laboratory service for Ancon Hospital as well as to serve as a Public Health Laboratory for the Canal Zone and the Republic of Panama. This laboratory, until then known only as the "Panama Laboratory", was described by Gorgas in the *Medical Record* of February 4, 1905, as follows: "In the grounds of Ancon Hospital we have a general laboratory under the direction of Doctors Herrick and Kendall, both Johns Hopkins men, well equipped for the work and for such laboratory investigation as is cognate to sanitary work . . . we hope to develop the laboratory work into a more general field of tropical investigation, and in this way we have a great hope of doing something that may be of general interest to the medical profession at large".

Doctor A. B. Herrick was the first Pathologist and Dr. Arthur I. Kendall his assistant, but although autopsies began on their arrival early in 1904, the laboratory functioned with limited facilities and even without a microscope for several months. The I.C.C. finally authorized the following personnel at the salaries stated, plus board:

1 Chief of Laboratory	$4,000 per annum
1 Pathologist	$3,000 per annum
1 Bacteriologist	$3,000 per annum
2 Attendants	$ 600 per annum

Doctor Herrick was a highly versatile physician and from the beginning was actually chief of all professional services, and highly respected for his ability. As the staff at Ancon grew he was promoted on December 27, 1905, to the rank of Chief of Clinics at a new salary of $4,500, but without board! His duties as Chief of Laboratory did not actually terminate, however, until June 1, 1906, when he became Chief of Surgery, a post he held for the next nine years. During this period (1906–1916) he contributed numerous monographs on the surgical aspects of amebiasis, appendicitis, hernia and other clinical entities.

Dr. Samuel Taylor Darling arrived on the Isthmus in February 1905 to be assigned as pathologist at Colon Hospital. In November 1906 he succeeded Dr. Herrick as Chief of the Board of Health Laboratory. He remained with the Isthmian Canal Commission until 1915, contributing more than 75 papers to various medical and parasitological journals during these 10 years as its most prolific investigator. He made important contributions to the scientific knowledge of trypanosomiasis, malaria, amebiasis and hookworm disease. He introduced the Wassermann test very early in the Canal Zone. In 1909 he identified a trypanosome disease fatal to American horses and mules, established its specificity by isolating a new trypanosome, *T. hippicum*, and described its mode of transmission. Subsequently he checked an epidemic of this disease, known locally as

murrina or derrengadera, by applying to animals some of the methods that had been used in Panama to control yellow fever and malaria. His studies on amebiasis did much to help clarify the true status of *E. histolytica*. He was an amazingly scientific and meticulous research worker. In one of his classic experiments he was seeking the dosage of gametocytes necessary to infect a mosquito, and by the ingenious device of weighing mosquitoes before and after their blood meals, determining at the same time the number of crescents per cubic millimeter of peripheral blood in the subjects on whom the mosquitoes fed, he showed that it required from 25 to 50 gametocytes of *P. falciparum* to infect one female *Anopheles albimanus*.

Perhaps his most famous discovery was the disease histoplasmosis. Within a relatively short time in 1905–06 he autopsied three individuals who presented a pathologic picture resembling that of kala azar, and after differentiating the causative organism from the leishmanias, described it as *Histoplasma capsulatum*. He then described the pathology of this new disease. The clinical picture was not well identified until much later, since only one of his patients had been under medical observation for more than a few hours. This discovery was not overlooked, but its importance was not fully realized until case findings of the disease were reported in other parts of the world and again, very recently, in Panama.

Among Darling's colleagues were George Ridley, Pathologist, 1906–07; George H. Whipple, Pathologist, 1907–08, now Professor of Pathology at the University of Rochester School of Medicine after serving many years also as Dean; Francis P. McCarthy, Pathologist, 1908–09; Arthur I. Kendall, Bacteriologist, 1904–06, who became Professor of Bacteriology at Northwestern University School of Medicine; Lewis B. Bates, Bacteriologist, 1909–1918, and successor to Darling as Chief of the Board of Health Laboratory 1919–1948; Herbert C. Clark, Pathologist, 1909–1917 and 1919–1922, a world renowned authority on tropical diseases, who became Director of Gorgas Memorial Laboratory in 1929; and outstanding technical specialists such as James Zetek, L. H. Dunn, J. B. Shropshire, J. E. Jacob and A. H. Jennings.

This organized medical department was effective in controlling disease in spite of the many difficulties, the most important being government red tape. A present-day government worker who feels he is burdened with procedural matter would be appalled at the hurdles which had to be overcome by Gorgas's staff to obtain equipment. Under the first Isthmian Canal Commission, medical supply was always too little and too late. Gorgas thus described his dealings with Admiral Walker, Chairman of the first I.C.C., regarding requisitions: "Day after day I would go to the Admiral with requisitions for various things needed and we would talk the matter over. He would always get on the subject (of economy) 'Gorgas there is one thing certain: whether we build the canal or not we will leave things so fixed that those fellows up on the hill can't find anything in the shape of graft'. After this he would take my requisition and stick it in a drawer where it would remain for an indefinite time". A typical example of the results of such economy occurred when a psychotic mother delivered a child and was unable to nurse it. The ward nurse applied to Major LaGarde for a

bottle and nipple. The request could not be filled since the nursing bottles ordered five months previously had not arrived. LaGarde prepared a special requisition, took it to Gorgas for endorsement and while the hungry child waited it was sent to the Chief of the Bureau of Materials and Supplies for his endorsement. Thereafter it had to be copied and embossed and several weeks later the item was obtained at a total cost of $6.30.

The basic problem was that while Gorgas was given a free hand in Cuba and accomplished his mission promptly, in Panama he was subordinate to many (13 at one time) who felt that the mosquito theory of disease transmission was a hoax. Because of this, the lack of supplies, and the influx of thousands of nonimmunes, a yellow fever epidemic started during the first 6 months of 1905 and nearly ruined the canal project. Panic resulted and more than 90 per cent of the U. S. employees left Panama in fear of the disease. Gorgas was constantly being sniped at and his resignation requested. The American Medical Association sent Dr. Reed to Panama who inspected the problems and reported his findings in the Journal of the American Medical Association, completely vindicating Gorgas and pointedly accusing the higher echelons as the cause of the continuance of yellow fever in Panama. The Canal Commission was reorganized and Gorgas remained but received no better cooperation, and in eight months time the Commission was again asking for Gorgas' resignation. On this occasion the request was approved by Secretary of State Taft and was sent to President Roosevelt who seriously considered his removal. However he first consulted Dr. William H. Welch of Johns Hopkins and Dr. Alexander Lambert, his personal physician, who said, "I am sorry for you tonight Mr. President. You are facing one of the greatest decisions of your career. Upon what you decide depends whether or not you are going to get your canal. If you fall back on the old method of sanitation you will fail just as the French have failed. If you keep Gorgas and his ideas and let him make his campaign against the mosquito, then you get your canal". The President was convinced and ordered the entire Commission to give its wholehearted support to Gorgas. John Stevens, Chief Engineer and always a backer of Gorgas, put the entire construction force at his disposal and all work was focused on yellow fever.

With the death of Joseph J. Trixler in October, 1905, the last fatality from urban yellow fever known to have been acquired in Panama was recorded, and aside from an ill-defined case in May, 1906, none has originated there since then. Only imported cases were encountered and yellow fever had been almost forgotten, although constantly watched for, until the outbreak of the sylvan form in the Republic of Panama in 1948. Gorgas was now able to devote his undivided attention to malaria and the control of its anopheline vector. Malaria had attained a peak incidence of 826 hospital admissions per 1000 employees in 1906, but after concentrating all sanitation on the control of anopheline breeding a rapid decline took place until by 1913, the rate had dropped to 70 hospitalizations per 1000 employees.

Next to Gorgas the outstanding sanitarian of the early days was Dr. Henry R. Carter. He had done extensive work with malaria and yellow fever, determining

the extrinsic incubation of the latter before the vector was discovered. Reed said, "his work did more to impress me with the importance of an intermediate host in yellow fever than everything else put together". He also set up the modern system of quarantine. To him and Gorgas go the credit for sanitating the Canal Zone. In addition he served as Director of Hospitals in the Zone and perfected the smooth running hospital system that cared for the workers during canal construction. He divided the Canal Zone into 20 districts, each with its medical officer, dispensary and small receiving hospital. He had a hospital train running both ways daily to the large hospitals in Colon and Ancon. The hospital cars were equipped with a medical officer, attendants and drugs, and were arranged with berths that could be raised and lowered, serving as stretchers so that patients could be moved with a minimum of discomfort. In addition to these facilities the Canal Zone ran a leprosarium, a hospital for the insane, and a convalescent ward on Toboga Island. The Republic of Panama paid the I.C.C. $.75 per day per patient and all Canal Zone employees and their families paid $.30 a day. Interns were paid $1200 per year and staff physicians $1800, $2400 and $3000.

Among others Drs. W. E. Deeks and W. C. James made history in tropical medicine during their stay in the Canal Zone. They both were among the early arrivals and Deeks served as Chief of the Medical Clinic with James under him. These two were clinicians but set up a small laboratory in Section C to study their own malaria slides and stool specimens. They wrote extensively on the various clinical pictures presented by patients with amebiasis. At a time when most dysenteries were carelessly called amebic and all amebas were felt to be pathological, they stressed and taught that 20–30 per cent of cases of amebiasis were latent and without specific symptoms. They studied the life cycle of *Endamoeba histolytica* and published their opinion that this was the only ameba pathogenic to man. They showed that all other pathogenic forms (and there were many at that time with a confused nomenclature) were in actuality only various stages of the life cycle of *E. histolytica*. Unknown to them a similar study had been published by Walker in Manila a year or two previously who rightfully gets credit for this observation. Deeks was the champion of the therapy of amebic dysentery with his regimen of milk diet, rest and massive doses of bismuth subnitrate. His technique was developing wide acceptance just before emetine was discovered. Much was also learned about malaria by these workers. At that time blackwater fever was not definitely identified with malaria, but these authors consistently showed that Dr. Darling was able to find parasites in their cases 100% of the time. In addition to tropical disease Deeks was much interested in carbohydrate metabolism and was often quoted in the stateside journals on his "Carbohydrate Diathesis".

Between 1915–1920 there developed a new phase in Canal Zone medicine. World War I caused vast numbers of army personnel to arrive on the Isthmus and many of the original physicians went elsewhere. Gorgas left and became Surgeon General of the U. S. Army. Darling was appointed a member of the International Health Board of the Rockefeller Foundation. Herrick, James and

Reeder went "across the street" and formed the Herrick Clinic and *Hospital de Panamá*. Deeks left in 1915 to become Medical Director of the United Fruit Company, and Clark went to the United Fruit Company as a pathologist at the same time.

INTERMEDIATE PERIOD: 1920–1940

What might be termed the "modern era" of medical activities in the Canal Zone (and in fact on the entire Isthmus of Panama) began between 1915 and 1919, when the old, unscreened frame buildings of the Ancon Hospital, built by the French in 1883, were replaced by attractive, reinforced concrete structures. Among the more important changes made, according to Col. Weston P. Chamberlain, were the building of a dental clinic, a new medical clinic, an increase in the number of private rooms, and a large addition to the nurses' home. Located "amid a veritable microcosm of beautiful and rare tropical plants, trees, and shrubbery" on the slopes of Ancon Hill and overlooking Panama City and the Bay of Panama, the new hospital afforded a beautiful view of the mountains and savannas in the distance, and prompted Col. L. T. Hess in 1922 to describe it as a quiet, insect-free haven where patients might convalesce with "ideal surroundings which act as a panacea for restoration to health". With a normal capacity of 800 beds and facilities for conversion to a 1200 bed hospital in the event of an emergency, its name was changed to Gorgas Hospital by a joint resolution of Congress in 1928. It was prepared to treat all types of illnesses and all classes of patients with the exception of the mentally sick, who were cared for at Corozal Hospital three miles away.

During 1920 an epidemic of influenza reached Panama, vividly described by Dr. Roland C. Connor. Invading the Isthmus during the 1st week of March, it appeared that the disease had spread from Chicago where it had been noted 6 weeks earlier. From March 1 to June 8, 1920, 601 patients, of whom 33, or 5.4 per cent, died, were admitted to the hospital with this diagnosis. Dr. Lewis Bates and Dr. Joseph St. John made attempts to culture the meningococcus from 400 of these patients and failed to recover this organism from a single case. They noted, parenthetically, that the Canal Zone had always been unusually free from meningococcus meningitis.

Stressing the relationships between disease and world economics, and pointing out the economic advantages to shipping firms of keeping their ships disease-free, Dr. Wm. C. Rucker outlined the maritime quarantine policy of the Panama Canal, namely: 1, to prevent the entrance of disease into the Canal Zone by (a) excluding it by inspection, disinfection, and the prevention of the landing of infected persons, animals, and things; (b) cooperation with other countries; and (c) stimulating other countries and the transportation interests to take those precautions which would prevent the embarkation of disease for canal ports; 2, to inhibit the world spread of disease by ships by (a) removing and destroying disease on passing ships; (b) stimulating the sanitary authorities of other ports to prevent the movement of disease from and to their ports; (c) requiring that vessels take precautions at other ports which would prevent the

transshipment of disease vectors; and (d) the continued education of the shipping interests regarding the potentialities of disease distribution by ships.

In January, 1921, Dr. Lewis B. Bates reported 3 autopsied cases of blackleg (symptomatic anthrax, or black quarter) on the Isthmus. This was the first time this disease had ever been observed in human beings. In October of that year Dr. Leland L. Chapman reported to the Medical Society of the Isthmian Canal Zone 19 autopsied cases (over a 6 year period) of infantile beri-beri similar to those described by Hirota in Japan 33 years previously and more recently by José Albert in the Philippines.

Following Darling's studies which showed the rat to be a disseminator of relapsing fever in Panama and the work of Lawrence Dunn on the tropical bedbug in relation to its transmission, Bates and St. John in collaboration with Dunn proved by human experimentation that the human tick, *Ornithodorus talaje*, is the transmitting agent of the relapsing fever in Panama and produced evidence to show that the relapsing fever spirochete (*Borrelia*) of Panama is a distinct species for which they later suggested the name, "*Spirochaeta neotropicalis*".

Along with a beautiful review of the epidemiology and clinicopathologic aspects of the disease, Dr. Roger Brooke reported in 1927 the first case of kala azar in Central America (although mucocutaneous leishmaniasis was already well known in Central and South America). Although the classical clinical features were present in this case, the diagnosis was by splenic biopsy and the patient was successfully treated with tartar emetic.

In the same year Stewart and McNabb showed in actual field experiments that while quinine will not prevent overt plasmodial infection in 50 per cent of men exposed, it suppressed clinical symptoms (and was thus of military value) in 94 per cent of the men.

Dr. Dalferes P. Curry, who in 1920 had recommended screening of buildings and education of the people in malaria control, in paying tribute to Col. Joseph Siler at the time of the latter's retirement as Chief Health Officer of Panama (1934), cited improvements in malaria control brought about by ditching, the spraying of breeding places of mosquitoes with Paris green from airplanes, and improvements in sewage and garbage disposal (building of a large open grate incinerator near Summit, 11 miles out in the country, to take care of garbage from Panama City, Ancon, Balboa, and Ft. Amador) and vividly described the smallpox epidemic of June–October, 1929, brought under control only by a vigorous vaccination program. Only one death resulted (from lobar pneumonia) among 339 cases in Panama City, 28 in Colon, and 5 in the Canal Zone.

While working with the reduviid bug, *Triatoma megista*, in 1907 in Brazil, Carlos Chagas discovered *Trypanosoma cruzi*. During the 20 years which followed 1909, when Chagas published his observations, the disease was also found in other South American countries and in El Salvador in Central America. In Panama the first recognized human case of Chagas' disease occurred in 1930 and together with two additional cases was reported in 1931 by Miller. In January, 1932, during a study of thick blood films taken for a malaria survey

Clark and Dunn found and reported 2 more cases of infection of man with *T. cruzi*. By the middle of 1934 Clark had collected records on a total of 12 human cases of Chagas' disease, all in the Pacific sector of Panama.

The first recognized fatal case of Chagas' disease in Panama was reported by De Coursey in 1935. The case was that of a 3 month old negro baby who died in August, 1933, and the diagnosis was made at autopsy. In this case trypanosomes in their leishmanial forms were found in the myocardium and in the brain causing death from a severe diffuse myocarditis and a widespread focal encephalitis. By March, 1936, the total number of reported cases of Chagas' disease in Panama was raised to 19 by the report of Johnson and DeRivas who added 6 new cases including 2 fatalities to the 13 previously recorded. In 1950 Wm. F. Enos and Norman W. Elton reported the first fatality from Chagas' disease in a North American in the Canal Zone who was a member of the Armed Forces and had been on sentry duty behind the Post of Corozal. It is of further interest that during the past few months pathologists working with Colonel Francis E. Council, Ferruccio Bertoli and Carl M. Johnson at the Board of Health Laboratory, in reviewing all autopsies in which non-specific acute myocarditis had been diagnosed throughout the years, have found *T. cruzi* in the heart muscle in many cases not previously recognized. Three of these occurred from 1920–1930 before the first fatal case was reported by De Coursey.

Dr. R. A. Kelser in 1936 devised a complement-fixation test for Chagas' disease using an antigen prepared from cultures of *T. cruzi*. This antigen represented a distinct improvement over the older types prepared from the hearts and spleens of artificially infected animals and no cross reactions were noted to occur with Wassermann-positive sera. Using this antigen Johnson and Kelser in 1937 conducted a survey of the incidence of Chagas' disease in Panama as determined by the complement-fixation test and found that the disease was more common than suspected, with an incidence as high as 20 per cent in some communities. The infection rate (as determined by the test) was low for children under 15 years of age but rose sharply above that age, due probably to a relatively high mortality from the disease in children. The authors concluded that the complement-fixation test is of distinct value not only in identifying active cases but in revealing the over-all incidence of this infection, past and present.

Among the outstanding contributions to the field of tropical medicine during the two decades under discussion were the experiments in malaria control conducted by the Army Medical Research Board in 1935–1936 under the direction of Lt. Col. James L. Simmons. This group presented convincing evidence to show that *Anopheles punctimacula* and a similar species, *Anopheles neomaculipalpus* (discovered by Dr. D. Currey in 1931) were possible vectors in the transmission of malaria. Moreover these species had different breeding habits from those of *A. albimanus*, the most common malaria vector on the Isthmus. Their observations called attention to the fact that the methods for control of *A. albimanus* were not applicable to these other species and they accordingly introduced an important practical problem in mosquito control for solution by the Sanitation Department.

No historical review of this type would be complete without a few words

about leprosy, that peculiar disease which has plagued mankind at least since biblical times. Dr. Kenneth O. Courtney traced its origin in Panama to the first Spanish settlement, Nombre de Dios, in 1509 and concluded that in the majority of cases the spread of this disease has been along the routes of territorial invasion and commercial travel. He thought of leprosy as a familial disease. At the time of his writing (1939) residual endemic foci of leprosy in the Republic of Panama existed in the towns and villages of Las Tablas, Los Santos, Chepo, Taboga, Bocas del Toro, and probably Marcia and Las Delicias. Anderson and Hurwitz meanwhile had written a beautiful history of the origin and development of the Canal Zone's Palo Seco leper colony and described the treatment and fatty acid research performed there. Childress and Kean contributed an analysis of the autopsy findings of 103 lepers in Panama.

In conclusion it should be pointed out that the above resumé constitutes but a small portion of the experimental work performed in the Canal Zone during this period. Among other studies performed were those on nutrition by Dr. Roger Brooke; De Coursey's analysis of 345 necropsies on patients dying from coronary occlusion; Dr. Herbert Clark's tick-biting experiments in bovine and cervine piroplasmosis; renal anomalies disclosed by 4,215 consecutive autopsies and the distribution and complications of amebic lesions found in 186 post mortem examinations; Denton's studies on the pathology of pellagra and fatal measles; the transmission of equine encephalomyelitis in Panama by *Aedes taeniorrhynchus* by Kelser; St. John's studies on the Wassermann reaction in malaria; the parasitic index of the school children of Panama City by Caldwell; Makel's report on ainhum; Feder's intestinal amebiasis from the pathologist's standpoint; Dart's report of 7 cases of sudden death from asphyxia due to acute non-diphtheritic inflammatory edema of the larynx; Bates and Connor's clinical and bacterial analysis of bacillary dysentery cases in the old Ancon Hospital 1919–1923, and many others.

RECENT PERIOD: 1940–1954

With the coming of World War II the importance of the United States tropical disease center in the Canal Zone became clearly apparent once again. Past accomplishments were remembered; immediate contributions were expected. For the first time the United States was fighting in the tropics. This meant many new problems. Several questions had to be answered. Japanese monopolization of quinine supplies had made the use of other antimalarial drugs imperative. New therapeutic agents had to be tested.

Col. Gorgas and his staff had cleared the Panama Canal Zone and its terminal cities from malaria and yellow fever. They had shown that adequate sanitation in large tropical areas was practicable. Furthermore it had been done quickly. These lessons were remembered when the war crisis arose and were quickly applied wherever needed in the tropical war zones.

With the war Panama became a great troop center. Many soldiers were stationed there permanently for defense; for numerous others it was only a temporary stop-over. But for all it was a new experience. The tropics were

completely foreign; one had to know how to survive. An especially important contribution at this time was the classification of all the injurious plants of Panama as well as those of food value by Paul H. Allen. This was to be of much help elsewhere in the tropics.

Several disease complexes were studied ranging from dengue and dengue-like fevers and their transmission, to toxoplasmosis, leishmaniasis and histoplasmosis. Notable work was done on all the latter. The concept of human chronic toxoplasmosis was established by Wray J. Tomlinson in a paper published in 1945 reporting the first such case in the literature. Ben H. Kean and Robert Grocott reported the first case of congenital toxoplasmosis from the Isthmus of Panama in 1948. Frank Mantz Jr., Harry R. Dailey, and Grocott in 1949 reported two more cases. Studies were also done on the occurrence and transmission of toxoplasmosis in canines by Grocott.

The problem of leishmaniasis was also greatly clarified. J. Snow, E. M. Satulsky and B. H. Kean described in detail the histopathology in one of the first English accounts. This was later amplified more completely by David B. Thornburgh, Carl M. Johnson, and Norman W. Elton. Then last year Carlos Calero M. and Johnson reported 25 new cases, thus proving that it was much more common in Panama than had been thought. Since Darling first encountered at autopsy three cases of histoplasmosis in 1905 the fungus was not seen again locally until Tomlinson and Grocott recognized a canine infection. Then after nearly 45 years without a single human case, 3 more were autopsied within a two-year period. The fourth case was reported by John H. Draheim, John R. Mitchell and Norman W. Elton in 1951. Investigating this great paradox a year later Harold Tucker, skin testing 1000 persons with histoplasmin, found roughly one-third to be positive. This proved histoplasmosis to be much more prevalent here and defined Panama as an important endemic center. Recently the fungus has been found in soil samples on the Atlantic side.

In the study of rheumatic fever in Panama Maurice Hardgrove, Lamont Whittier and Elmer R. Smith reviewed the admissions at Gorgas Hospital since the early 1930's and proved rheumatic fever to be not uncommon in Panama, contrary to the opinion of the day that the disease was rare in the tropics.

Plasmoquin in the treatment of malaria was known to have toxic effects, but its use was still popular in the early 40's. Then in the month of May, 1943, 385 cases of proven plasmoquin toxicity were admitted to Canal Zone hospitals. This study by Maurice Hardgrove and Irving L. Applebaum markedly curtailed its use except in very selected cases.

Another significant contribution of this era was a study on the problem of intestinal helminthiasis in children by Nathan H. Einhorn, J. F. Miller and Lamont Whittier. They reviewed over 600 cases and re-evaluated therapeutic agents in the control and eradication of intestinal helminths.

Ainhum, the exotic malady of West Indians, was extensively studied in all aspects by Kean, Tucker and William C. Miller, and later by Butz and Elton. In fact most of what is known about ainhum in Panama came as a result of these studies.

Another interesting study by Kean was on the incidence of hypertension among the Cuna Indians, a primitive Indian tribe of eastern Panama. He found it almost non-existent. Then Horace P. Marvin and Elmer R. Smith found the incidence of hypertension much greater in West Indian negroes than in Panamanians, probably attributable to the predominance of Indian blood in Panamanians.

In 1952 Howard and Meriwether reported on the very interesting subject of fatty disease of the liver in infants. This revealed the existence in Panama of an unusual nutritional syndrome related to kwashiorkor in Africa.

In 1950 Enid C. de Rodaniche and Arcadio Rodaniche of Gorgas Memorial Laboratory and Santo Tomás Hospital isolated a strain (AS) of *R. rickettsii* from a native Panamanian who died and from another who recovered, thus identifying spotted fever for the first time on the Isthmus of Panama. In 1952 John H. Draheim and Enid de Rodaniche reported a case of herpes simplex encephalitis, with isolation and identification of the virus. In 1953 Michael J. Takos and Norman W. Elton reported spontaneous infections with *Cryptococcus neoformans* in the marmoset monkeys, *Leontocebus geoffroyi*, of Panama, adding a third lower animal host species to those previously encountered (horse and cheetah).

Perhaps the most notable achievement of the recent period came right after the war. This was the study of the pathology and epidemiology of jungle (sylvan) yellow fever by Norman W. Elton and his group at the Board of Health Laboratory. The jungle form of yellow fever, commonly called woodcutter's disease, had been found to exist in eastern Panama in 1937. During the period of World War II the Panama Mobile Force maintained base camps and outposts throughout central Panama, one of its principal missions being to keep close contact with the rural population and their diseases. At this time all United States troops were immunized against yellow fever, but the Panamanian people were not. No evidence of the disease was observed. Then, late in 1948, jungle yellow fever killed five farmers in the Pacora District a few miles east of the Canal Zone and west of the known enzoötic area in eastern Panama. Vaccination programs for the general population were immediately initiated on an extensive scale. Within the following year more deaths were diagnosed from localities further west along the transisthmian highway close to the Canal itself. In January, 1950, a fatality was diagnosed 12 miles west of the Canal on the Atlantic side, confirming Elton's ever mounting suspicion that a moving wave was in progress. With this evidence he postulated that this wave was advancing westward through the Atlantic rain-forest of Panama. On March 20, 1950, the Minister of Public Health in San José, Costa Rica, was alerted to anticipate involvement of the Atlantic side of that country within 14 to 18 months, and in fact the first death from yellow fever in Costa Rica occurred in the vicinity of Puerto Limón in July, 1951. But because of vaccination the human toll was light in contrast with what it might have been. The wave continued to sweep inexorably onward, with its initial epidemic center in Nicaragua in the Mico Valley in July, 1952, as had again been predicted by Elton. It then passed northward up the Atlantic

watershed of that country and in January, 1954, precisely on schedule at an overall velocity of 13 miles per month, had invaded Honduras with its initial epidemic outbreak in the Guayape Valley in the region of Juticalpa, Catacamas and Culme. Contact with the wave has steadfastly been maintained and vaccinating teams have consistently preceded its arrival. This has meant enormous effort and endless watching . . . but the reward has been great. Much human mortality and morbidity have been prevented.

And so another chapter in preventive medicine has been written and a battle won. How many more lie ahead of us still to be fought? Let us hope that historic-mindedness will prevail over present-mindedness and that insufficient support may not impair the effective operation of this Gorgas Center that has so commendably typified United States effort in making a large area in the tropics habitable by men of every race. The Canal Zone has been since its inception an experimental community in preventive medicine and public health. May it continue to be a wakeful giant measuring up to the task of furthering our understanding of the problems of tropical disease.

NUTRITION PROBLEMS IN TROPICAL AREAS

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There is very little exact information on nutritional requirements in the tropics. Man seems to have prospered and to have attained a better state of well-being in the regions of temperate climate, and these are the areas where his alimentary needs have been intensively studied. Studies so far indicate however that the nutritional requirements, except as noted in a later paragraph, do not differ very greatly from temperate zone standards. Among recent articles concerned with the problem of tropical nutrition should be mentioned those by Van Veen (1951) and by Mayer (1951).

The tropics usually involve high temperature and high humidity, both of which not only influence physical comfort and physical activity, but also provide an environment characterized by increased microbial and parasitic activity. These in turn are related to ill health and malnutrition. There are also certain specifically nutritional diseases which flourish in the tropics. We are commenting briefly on some of these diseases:

KWASHIORKOR

Kwashiorkor is widespread in tropical areas and has been studied particularly as it occurs among the non-white population of equatorial Africa. The disease has been described in some detail by Brock and Autret (1952), by Dean (1952) and by Trowell (1952). A similar condition has also been reported from Egypt by Hanafy (1947), and from India by Achar (1950). Kwashiorkor is seen in newly weaned infants and in children up to 3 or 4 years of age, and is characterized by changes in skin similar to pellagra and by graying of the hair, low serum albumin, edema, fatty liver, and a high mortality. The disease responds quickly to a good protein diet, particularly when milk is fed. A similar syndrome has been reported for adults in areas where kwashiorkor is common.

Kwashiorkor would seem to be common in areas where the diet is low in protein and predominantly of the maize-yam type. That it is a nutritional deficiency disease is now generally conceded, but it may well be the result of multiple deficiencies, of which a lack of good protein is the most important. The situation is often complicated by a high incidence of intestinal parasites. While the feeding of milk seems to be the best corrective for this disease, the fact that milk is not readily available in many areas has stimulated a search for effective milk substitutes of plant origin.

Kwashiorkor is but one variety of infant malnutrition in the tropics which is being investigated. Others include mandanna in Ceylon, diboba in the Congo. The term malignant malnutrition has been used at times to include all of these. It is even possible that these names all refer to the same malady. The factors involved are many and the syndromes, where they are referable to deficiencies,

certainly are not those of simple deficiencies. They all need more detailed study. A recent review by Oomen (1954) on infantile malnutrition in Indonesia is of interest.

BERIBERI AND PELLAGRA

Beriberi and pellagra are the classical vitamin deficiency diseases characterized by deficiencies of thiamine and of niacin respectively. Their cause, lesions, methods of treatment, etc. have been studied in considerable detail and are adequately discussed elsewhere.

TROPICAL SPRUE

The cause of tropical sprue is uncertain. It seems to be associated with deficient diets and responds to folacin, and to vitamin B_{12}. The disease is characterized by low absorption coefficients, particularly with respect to fat, but it may be accompanied by other effects, namely diarrhea, anemia, glossitis and loss in weight. While it certainly shows a nutritional relationship, some regard it as fundamentally an infectious disease. It is not found in all tropical areas, being notably absent from central Africa. Manson-Bahr (1953), formerly a proponent of its nutritional character, has more recently suggested that a virus may be responsible for tropical sprue.

TROPICAL ULCER

Tropical ulcer, previously regarded as an infectious disease, is now generally accepted as a nutritional disease. The studies of Charters (1943) in East Africa indicate that a deficiency of vitamin A in the diet is the important factor.

OTHER DEFICIENCY DISEASES

The above list of the deficiency diseases of the tropics is certainly far from complete. While the study of the deficiency diseases has so far been pursued more vigorously in the temperate regions, it is probable that many of the vitamin deficiency syndromes will only become manifest under the unusual stress of climate and nutritive imbalance, conditions which the tropics provide. Those of us who experienced the unnamed nutritional difficulties of the civilian internment camps in the Far East during the last war are convinced that further advances in our knowledge of vitamins and the nutritional deficiencies generally may be expected to follow upon more thorough study of the problems of nutrition in the tropics.

RESISTANCE TO TUBERCULOSIS, ETC.

The relationship of malnutrition to certain infectious diseases is so pronounced, particularly in the tropics, that these almost become nutritional deficiency diseases. Tuberculosis is one of the scourges of the tropics. Those who have observed the prevalence of tuberculosis in China and the salutary results which follow improvement in the diet, do not hesitate to list tuberculosis as one of

the diseases most intimately related to nutritional status. It should receive more than passing mention when discussing nutrition in the tropics.

HUMAN NUTRITION IN RELATION TO HELMINTHS

In tropical areas, infestation with intestinal parasites is common. In nutrition studies with human subjects in the Far East our routine procedure included the administration of an anthelmintic, and our experience invariably indicated this was necessary. There is now increasing evidence that deficient diets generally benefit the helminth. Experimental data has been reviewed by Hunter (1953) who notes that this is the case with diets deficient in vitamin A, with those deficient in vitamin B-complex, and with diets both quantitatively and qualitatively deficient in protein. This may be explained by supposing that the deficient diet leads to a weakening of the host's resistance to the helminth. On the other hand, experimental results indicate that helminths flourish on a diet rich in starch; and this is of course the type of diet which is characteristic of many tropical areas.

CALORIC REQUIREMENT IN THE TROPICS

It is generally accepted that a colder environment means a higher total caloric requirement and in the same way a tropical temperature requires less calories; quantitative data on this relationship however, particularly in respect to hot environments, is not very plentiful. Among recent studies is that of Johnson and Kark (1947) who measured voluntary caloric intake of military personnel assigned to different world areas. Their data indicate a relationship between daily caloric intake and what they term mean annual external temperature which is expressed by the following equation:

$$\text{Calories} = 4151 - 28.62T$$

where T is the temperature expressed in degrees centigrade. The Food and Agriculture Organization of the UN (1950) has suggested that for approximate calculations the standard figure for caloric requirement should be reduced by 5 per cent for every 10° of mean annual external temperature above the reference temperature. This would seem a safe guide, but it must be understood that it is only approximate and does not take into consideration other factors in the environment. Moreover this simple calculation holds only for individuals engaged in similar types of muscular activity. There is a scarcity of data which can be used for calculating the caloric requirements of non-white populations in the tropics.

Some of the tropical peoples are of smaller stature; a smaller body size almost always means a reduced caloric requirement, other factors being equal. Most of our standards have been fixed with respect to a reference man 25 years old and either 65 or 70 kilos of body weight. When it becomes necessary to calculate the calorie requirement for an adult of different body weight, this is usually carried out using some form of the general equation:

$$\text{Calories} = aW^{0.73}$$

where W is body weight and a is a constant. For example, if the calorie requirement for the standard adult man of 65 kilo body-weight is 3200 calories, this becomes for the 50 kilo man, 2640 calories. Likewise if the standard adult woman of 55 kilos requires 2300 calories, the equivalent 45 kilo woman requires 1985 calories. These factors are discussed in some detail in the United Nations publication mentioned above.

Among all aspects of the caloric requirement, the factor of basal metabolism has been the most intensively studied. Early observers were intrigued by data which suggested that racial differences were revealed in these measurements. It may now be concluded that the basal metabolic rate of inhabitants of the tropics is on the average about 10 per cent lower than that of inhabitants of the regions with temperate climate. This is true apparently both for races indigenous to the tropics and, as shown by MacGregor and Loh (1941), for people from other areas who have become residents of the tropics. The effect of race and climate on basal metabolism has been discussed by Keys *et al.* (1950).

GENERAL NUTRIENT REQUIREMENTS

When nutritional science made its first contacts with the tropics, it was suggested that the nutritional requirements of tropical peoples might differ from those of other countries, that is, there might be racial differences in bodily metabolism. During the last two or three decades a large number of nutrition studies have been carried out in tropical areas. While many of the experiments reported are of short duration, it may be concluded that at least the requirements for the major nutrients, protein and calcium, do not differ significantly from the figures accepted in other countries for individuals of equivalent body weight.

Economic factors and the factor of population pressure itself are of overwhelming importance in the tropics. In many regions there is not enough land or agricultural product to meet the needs for food. Not only is the vegetarian diet the common diet, but malnutrition is widespread. While the nutritionist in the more favored areas of the earth's surface discusses optimal diets and optimum requirements, the nutritionist in the tropics finds it necessary to plan in terms of a subsistence level and to discuss minimum diets and minimum requirements. The National Research Council's "Recommended Allowances" which are widely used in the United States are frankly unattainable in many tropical areas and what is needed is a dietary schedule on a more practical level carefully keyed to the agricultural economy which prevails in the majority of these regions.

Nutritional data for China have been reviewed by Adolph (1946) and for India by Patwardan (1952). In considering nitrogen balances, it should be noted, as pointed out by Adolph (1944), that vegetarian diets invariably are accompanied by low protein digestibility coefficients. Scrimshaw *et al.* (1951) report high plasma protein levels in Central America, and are publishing significant data on other nutrients. Many more studies of this type are needed. Comprehensive nutrition surveys carried out in tropical areas should yield significant data.

Squibb *et al.* (1954) in the first of a series of studies on the nutritional consequences of high environmental temperatures report that with rats the level of serum protein is significantly increased at high temperatures; at the same time growth is depressed.

The smaller stature of some tropical peoples has been a confusing factor in all nutrition studies. When nutritional status has been evaluated in terms of height and weight, the height-weight standards of temperate regions have often been used. More complete height-weight tables for tropical areas are needed.

The smaller body weight in some of the peoples of the warmer regions is not easily explained. The peoples of northern India are on the average larger than the peoples of southern India and the same difference is found between the people of northern China and southern China. Is the diet responsible for this? The observations of McCarrison (1936) would indicate that it is. The suggestion has been made that the smaller skeletal development of the southern Chinese reflects a smaller calcium intake, but little evidence has been accumulated so far to support this. The anthropologists point out that in cold climates large body size aids heat conservation and in hot climates small size aids heat loss (Bergman's Rule).

VITAMIN NEEDS AT DIFFERENT TEMPERATURES AND VARYING HUMIDITY

Experiments with laboratory animals indicate that at different environmental temperatures the vitamin requirement is lower at the higher temperature. This has been shown for thiamine by Hegsted and McPhee (1950), for riboflavin by Mitchell *et al.* (1950), for vitamin A by Ershoff (1950), and for vitamin C by Dugal and Therien (1947). No corresponding data on these vitamins for humans are recorded as far as we know.

There have been very few studies on the influence of humidity on nutrition. Collins *et al.* (1953) observed abnormalities of the skin and fur of rats with vitamin deficient diets when the relative humidity was below 30 per cent and over 90 per cent. More data is needed on this factor. In China, of the two factors temperature and humidity, we felt that the well-being of the rats in our experimental colony was more intimately related to humidity.

SUGGESTIONS ON NUTRITIONAL IMPROVEMENT

Malnutrition is often accompanied by economic and sanitary problems. But from the purely nutritional standpoint the first emphasis should be placed on a supply of good protein to supplement the cereal diet. One of the shortcomings, if not the principal shortcoming, of cereal protein is that it lacks a sufficiency of the amino acid lysine; lack of tryptophane and of methionine may also lead to critical amino-acid deficiencies. The chemical industry has promised that lysine will soon be available for enrichment purposes on a large scale. This may go far in solving the problem of good quality protein.

The next point of emphasis should be a plentiful use of the "protective" foods, particularly green vegetables. In too many areas of the tropics these are neglected. In some areas farmers report that the green vegetable crops are easily

stolen and they therefore concentrate on cereal and root crops. In other areas farmers look down upon green vegetables as produce of lower value and of lesser prestige.

Where white rice is the staple cereal, it should be enriched with vitamins. An interesting experiment is now being conducted on the Bataan peninsula in the Philippines which is demonstrating what is possible with an enriched rice program in large-scale feeding. Where white flour is imported for large-scale feeding and for relief projects, the enriched variety should be used.

ANIMAL FOODS FOR TROPICAL AREAS

The same problems which face human health also face an animal industry in the tropics, that is, infection and parasitic infestation. The difficulties in introducing to the tropics breeds of cattle which have prospered in the temperate regions but when moved to tropical areas are confronted with varieties of parasites to which they are unaccustomed, are very great. Some success has attended the development of a meat and milk supply from animal species already available in the tropics. The carabao (water buffalo) for example yields a milk rich in fat, and its use as a source of animal food should be extended. However the problem of animal protein foods is not to be solved by simply increasing the animal population, for in many of the tropical areas the animal population is already very large. The problem is in fact much more complicated for it involves the question of forage and of the balance between forage and feed crops for animals and food crops for human consumption.

Tropical waters are rich in fish and many of the tropical peoples use fish in large amount. The fishing industry is capable of more intensive development and may be looked upon as a very practical source of animal protein. The European moving to the tropics usually takes his dried milk powder, canned butter and canned meat with him. A satisfactory food economy in the tropics however must ultimately rely upon indigenous sources for these nutrient items. When these foods are not obtainable, people revert to the high carbohydrate diet based on maize (mealies) and the high-starch root vegetables, and this is the picture of what in many areas has already happened.

REFINED FOODS AND AN EDUCATIONAL PROGRAM

Some of the malnutrition in the tropics is related to the introduction of refined foods, such as white flour, which are welcomed because they are convenient and found to be useful in projects which employ a large number of laborers. Experience indicates that the use of such food materials is successful when accompanied by an intensive educational program to enlighten people in the use of sufficient "protective" foods.

An interesting example of the disturbance which may accompany the assault of new popular foods upon the established nutritional status-quo is found in certain areas of the Near East where tea-drinking has become a wide-spread fad. In many of the areas concerned, the food budget is already limited, but, in spite

of this, tea which is expensive is replacing important nutrients in the dietary that the consumer can ill afford to lose.

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THE SIX-YEAR PHILIPPINE-AMERICAN MALARIA CONTROL PROGRAM

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No one knows for how many centuries malaria has plagued the people of the Philippines, but it is reasonably certain that the disease was indigenous in 1521 when Magellan went to the Islands (Russell, 1936). Soon after the American occupation in 1898 malaria was recognized as a problem and it has continued to be one of the most important diseases in the Philippines up to the present day, ranking as the number one cause of morbidity and among the top five causes of mortality. In 1935 Russell (1936) estimated that malaria was responsible for 10,000 to 20,000 deaths and at least two million cases of illness annually; at that time the population of the Islands was only about 13 million as compared with an estimated 21 million in 1953. In the traditional manner malaria rates increased during World War II, and at the end of the Japanese occupation it was estimated that there were over four million cases annually (Smith, 1950). Since that time there has been a gradual decrease in the incidence; for example mortality statistics for malaria show 10,558 deaths reported in 1948 and 7,170 in 1952. In some instances at least the decline in malaria has been correlated with a corresponding decline in the population density of the vector (Mendoza, 1953). The available data on morbidity and mortality are quite inadequate, but the general consensus is that malaria rates are now approximately at the prewar level. The latest surveys by the Malaria Control Division of the Philippine Department of Health show that in over half of the provinces malaria is hyperendemic in one or more areas. Of 4,505 barrios in which spleen surveys were conducted, six per cent were hyperendemic (over 50% positive), 31 per cent were mesoendemic (10 to 50%), and 29 per cent were hypoendemic (less than 10%).

Malaria imposes a great economic handicap upon the Philippines. The annual losses due to this disease are variously estimated at between 100,000,000 and 750,000,000 pesos. It is a rural disease and its effects are particularly severe in connection with agricultural, road and other economic development projects, the so-called "tellurism" of the tropics.

EPIDEMIOLOGY

Anopheles minimus flavirostris is the principal vector, and *A. mangyanus* and *A. maculatus* are considered as secondary vectors (Smith, 1950). All three species are stream breeders. *A. minimus flavirostris* usually breeds along the shaded margins of clear, cool, slow-flowing streams where bamboo roots, grass, or similar materials intercept the water surface (Ejercito, 1952). It is not uncommon to find larvae in other situations such as open rice fields or even in

warm or brackish water (Smith *et al.*, 1950); however, such findings are, in general, considered quantitatively unimportant though qualitatively interesting. *A. minimus flavirostris* generally does not breed in streams above 2,000 feet altitude, and malaria is chiefly limited to the foothill regions below this elevation, although there are occasional exceptions (Villanueva and Kalaw, 1953). In some instances of malaria transmission at higher altitudes, it appears that *A. maculatus* may be the vector (Baisas *et al.*, 1950). Flat coastal plains and marshes are essentially malaria-free, but where hills or mountains rise directly from the sea, coastal areas may be highly malarious.

Malaria in the Philippines is a rural disease, being most prevalent in barrios with populations of less than one thousand. As an area evolves from a rural to an urban community, *A. minimus flavirostris* generally disappears, probably due to clearing, pollution, or other changes in the streams in which it breeds. However, this is not invariably the case; for example, in 1931 *A. minimus flavirostris* was found breeding in a canal in the northern part of Manila (Baisas *et al.*, 1950), and 16 years later, in 1947, larvae, pupae and adults were collected from two streams in this same area (Dy and Rosario, 1948). In the fall of 1953 there was a small localized epidemic of malaria in Quezon City a little over ½ mile from the City Hall (Yogore, 1953). Carriers from nearby malarious provinces were apparently involved, but there was also indication of local transmission, and *A. minimus flavirostris* was found breeding in a small stream (Tanke Creek) running through the area.

Although it has been shown that *A. minimus flavirostris* can fly at least 2 kilometers, its effective flight range is generally not over one kilometer (Russell, 1936). During the daytime adults are usually found resting in cool moist dark harborages such as under the overhanging banks and vegetation of the streams in which the species breeds. It was originally believed that the species was not house-inhabiting, but careful inspections during the initiation of the Mindoro Pilot Project in 1952 showed that *flavirostris* did rest inside dwellings during the daytime with a fair degree of frequency (Bhatia, 1952). Subsequent observations in several other malarious areas of the Philippines revealed *flavirostris* resting inside houses during the day, a single inspection yielding as many as 43 adults (Baisas, 1953); in other equally malarious areas it is very difficult to find adults inside houses. Indoor-resting *flavirostris* are usually found on vertical surfaces within one foot of floor level and are rarely seen resting more than 5 feet above floor level (Baisas, 1953). Although the species readily enters houses at night to take human blood meals, it appears to be less domestic than *Anopheles minimus minimus*. As suggested by Laurel (1934) a number of years ago, there is a possibility that *A. minimus flavirostris* may consist of two distinct races, one more domestic than the other, but this has not yet been clearly established.

In those regions of the Philippines which have distinct wet and dry seasons, breeding of *A. minimus flavirostris* may be interrupted during the dry season by the drying up of small streams or during the wet season by the flushing action of heavy rains; thus, there may be two seasons of transmission, one at

the beginning and one at the end of the dry season. In those regions where there are no marked wet or dry seasons, malaria is perennial (Russell, 1936).

Although *Plasmodium malariae* and *P. ovale* are present in the Philippines, most of the malaria is caused by *P. vivax* and *P. falciparum*. Surveys by the Malaria Control Division during the past year indicate that roughly two-thirds of the positive blood smears are *P. vivax* and one-third *P. falciparum*, which is about the same proportion as reported by Russell in 1936.

MALARIA CONTROL IN THE PHILIPPINES

The early history of malaria control in the Philippines has been well summarized by Russell (1936), and a bibliography on malaria in the Philippines has recently been published by Walker (1953). During the first part of the century, control consisted almost entirely of the administration of quinine, which brought some relief but not control. The American Army used mosquito nets from the first days of occupation, and this has continued to be an important preventive measure to the present day. Gambusia top minnows were introduced from Texas to the Hawaiian Islands and thence to Manila in 1913, but have not had any detectable effect upon malaria rates.

Malaria investigations and control in the Philippines received a welcome stimulus in 1921 when the International Health Division of the Rockefeller Foundation took an active interest in the problem. Five years later, in November, 1926, there was established in the Bureau of Health a Division of Malaria Control, and for the past quarter of a century this organization has been responsible for developing information and procedures for the control of malaria in the Philippines. It was not until about this time that the Bureau of Health began to base malaria control on the fundamental premise that the vector was a stream breeder rather than a stagnant water breeder. This fact had been discovered by Whitmore at Fort Stotsenburg in 1904, lost in unpublished records for 10 years, rediscovered by Walker and Barber in 1914, and ignored for another 10 years (Russell, 1936). When the stream breeding habits of the principal vector finally received general recognition, attention was given to the use of larvicides, particularly Paris green which had been suggested for local use by Barber 10 years earlier. Paris green was first used with notable success at Fort Stotsenburg, malaria admissions at the hospital dropping from 415 in 1924 to 10 in 1930. This larvicide was subsequently used to good advantage in other local areas, but the method was not economically feasible for Island-wide control, and chemotherapy remained the only malaria control measure receiving widespread use.

About 1930 attention was first given to the use of naturalistic control measures, and during the next five years experiments were conducted with damming, periodic flushing, cutting vegetation to expose to sunlight, clean-cutting and sloping banks, ditching (channeling), packing with vegetation, and introduction of larvivorous fishes (Ejercito, 1936). The promising results achieved in these early studies led to more extensive field investigations, particular attention being given to the more permanent measures such as installation of automatic

siphons, clearing and terracing stream beds for rice culture (sanito-agronomic), and damming stream beds with subsequent use of the impoundment for the growth of fish or other products of aquiculture (sanitoaquinomic). World War II brought a disruption of this work during the 5-year period from 1941 to 1945, but it was given renewed attention during the post-war period (Ejercito, 1951) This resulted in the general conclusion that these permanent and semipermanent naturalistic measures were highly effective and that they would fit in with the long-range agricultural and regional development of the Philippines, but their use was not practicable for a rapid nation-wide reduction in the prevalence of malaria. The best modus operandi for malaria control under Philippine conditions therefore seems to be to "knock malaria down" through the use of less permanent and relatively inexpensive measures such as residual spraying with insecticides, and then to follow this up with a long range program of more permanent naturalistic measures aimed at "knocking malaria out" (Ejercito, 1952).

EFFECTIVENESS OF DDT AGAINST *A. MINIMUS FLAVIROSTRIS*

The discovery of the insecticidal action of DDT during World War II and of its effectiveness against malaria mosquitoes (Annand *et al.*, 1944) ushered in a new era in the history of malaria control. The dramatic results achieved with DDT in other countries led to an investigation of its potentialities in the Philippines early in the postwar period.

U. S. Public Health Service studies. Following the liberation of the Philippines, the United States Public Health Service carried out a "Philippine Public Health Rehabilitation Program" from July 4, 1946, to June 30, 1950 (Smith, 1950). As a part of this program, malaria control operations were carried out in about 20 malarious areas on Luzon, Mindoro, Marinduque, Palawan, Panay, Negros and Mindanao. Wherever circumstances were adaptable, the more permanent measures were utilized, such as automatic siphons, sluice gates, sub-soil drainage, stream straightening, etc; however, DDT was also used in several ways as a temporary control measure for *A. minimus flavirostris*. DDT-treated sawdust was found to be an effective and relatively inexpensive larvicide (Smith and Dy, 1947). DDT thermal aerosols applied by airplane for control of adults were found to be prohibitively expensive, but DDT fogs were applied routinely by ground equipment in a number of areas.

Preliminary experiments were also conducted on the effectiveness of DDT residual house sprays against *A. minimus flavirostris* (Smith & Dy, 1949). Five per cent DDT emulsion was applied at the rate of 2.0 to 3.5 gm. per square meter to 1750 houses and other shelters in three areas in the provinces of Laguna, Negros Occidental and Palawan in the fall of 1948. An appraisal of the results one year later showed that parasite rates in both children and adults had decreased in all three areas, the reductions ranging from about 6 to 85 per cent. Spleen rates in children 2 to 10 years old were lower in two of the three study areas, the decrease being about 97 per cent in one area. Unfortunately, circumstances did not permit the obtaining of infant parasite rates; the con-

trol areas were inadequate; the pretreatment endemicity was quite low in some of the areas; and observations could not be carried beyond one year. The results of the preliminary, experiments, while stimulating further investigation on this method of control; left doubt in the minds of the investigators and some others as to whether malaria could be controlled in the Philippines through the use of DDT residual sprays.

The Mindoro Pilot Project. During 1949 and 1950 a few small scale field observations on the effectiveness of DDT residual sprays against *A. minimus flavirostris* were made by five of the Field Units of the Malaria Control Division. The results were inconclusive, primarily because inadequate facilities limited the scope of the investigations. It therefore appeared that before the use of DDT residual sprays could be incorporated as a part of the modus operandi for a nationwide malaria control program in the Philippines, it would be necessary to conduct a careful field study on a sufficiently large scale so that fully conclusive results would be assured. Accordingly, a cooperative pilot project was established at Pinamalayan in a highly malarious area of Mindoro Oriental. In this project, WHO provided technical equipment, a malariologist who directed the project, and an entomologist (this team had conducted a similar project in Thailand). The Malaria Control Division of the Philippine Department of Health provided a malariologist, a medical entomologist, field personnel, and other peso costs; and the United States Government through MSA (now FOA) provided vehicles, sprayers, DDT, and other dollar commodities.

The area selected for treatment had a population of about 25,000 with some 5,000 houses to be sprayed; a comparable untreated comparison area was located to the north of the sprayed area. The study areas were highly malarious as indicated by the following data on spleen and parasite rates immediately prior to spraying:

	Sprayed Area	Comparison Area
Infant parasite rates	32	33
Spleen rates	77	57

The material used was a 75% DDT water dispersible powder applied as a 5% DDT water suspension at the rate of approximately 2.0 grams per square meter. Outbuildings as well as houses were treated. A detailed report on the project will be published by the investigators when the studies are completed; however, a preliminary report of observations made 11 months after spraying (Sambasivan *et al.*, 1953) indicates the following:

1. Malaria transmission (as measured by infant parasite rates) was effectively controlled in the sprayed area, while continuing high in the comparison area.
2. The spleen rate dropped from 77% to 32% in the sprayed area, with no significant change in the comparison area.
3. There was a significantly lower incidence of parasite positive fever cases in the sprayed area than in the comparison area.

4. *A. minimus flavirostris* adult densities dropped to and remained near zero in the sprayed houses while significant numbers continued to be found in the unsprayed houses of the comparison area.
5. The density of larvae and of adult *A. minimus flavirostris* in outdoor resting places was significantly lower in the sprayed area.

On the basis of their preliminary evaluation of results, the investigators made the tentative conclusion that malaria carried by *A. minimus flavirostris* can be controlled by one yearly application of DDT at a dosage of 2.0 gm. per square meter to inside walls and ceilings of houses. This conclusion is concurred in by the authors of the present paper, and has provided the basis for undertaking a nationwide program of DDT residual spraying. Those who conducted the Mindoro Pilot Project are to be commended for the careful and unbiased manner in which this extensive field demonstration was carried out. It marks a significant forward step in the history of malaria control in the Philippines.

THE SIX-YEAR PHILIPPINE-AMERICAN PROGRAM

Pending the outcome of the Pilot Project, Malaria Division and FOA personnel proceeded with the initial preparations for a DDT residual spray program. This included the establishment of a training curriculum for field personnel, the preparation of specifications and procurement requests for basic equipment and materials, and formulation of guiding principles. These preparations made possible greatly accelerated action as soon as the favorable results of the Mindoro Project became apparent.

By demonstrating the effectiveness of DDT residuals against *A. minimus flavirostris*, the Mindoro Pilot Project made it possible to proceed with the preferred modus operandi for malaria control in the Philippines—namely to "knock malaria down" with a short-range program of nationwide DDT residual spraying, and to "knock malaria out" with a long-range program of more permanent measures. Accordingly, the staff of the Malaria Control Division of the Philippine Department of Health and Technical Assistants of the U. S. Operations Mission to the Philippines developed a joint plan entitled "A Six Year Philippine-American Plan for Malaria Control in the Philippines" (Ejercito, Hess and Willard, 1953) which was subsequently approved by the representatives of the two governments and put into operation early in 1953. The nationwide operations are under the direction of the Chief of the Malaria Control Division of the Philippine Government, which provides technical staff, field personnel and other peso costs; and the United States Government through its Operations Mission to the Philippines which provides technical assistants and dollar commodities such as vehicles, sprayers, DDT, microscopes, and antimalaria drugs. The basic features of the Philippine-American Program are outlined in the following paragraphs.

Objectives. The two major objectives of the Philippine-American Program are:

1. During the period of 1953–1958 to reduce the prevalence of malaria in the Philippines to a level where it will no longer constitute an important public health problem.

2. To develop a long-range program of more permanent measures whereby the Philippine Government can, with its own resources, keep malaria under control and eventually eradicate it.

Methods of control. The primary method of control is the application to houses in all of the malarious areas of the Philippines of DDT residual sprays once each year for at least three consecutive years. After three years, area-wide coverage will be discontinued and spraying will be limited to situations where surveillance indicates that foci of transmission still persist. A unique feature is that after 3 years of spraying in the Mindoro area this principle of "interrupted spraying" will be applied on an experimental field demonstration basis for two years before it is applied on a nationwide basis (see Plan of Operations).

The formulation used is 75% DDT water-wettable powder, diluted with water to form a 5% DDT suspension for field use. In a few situations where the type of housing demands it, 5% solutions of DDT in fuel oil are used. Dieldrin, BHC, and other newer insecticides are being field-tested to determine if they are more economical and/or effective adulticides. The DDT residuals are applied with compression sprayers[1] of three gallons capacity (two gallons spray) equipped with the conventional 0.2 gm. fan-type spray nozzle.[2]

Antimalaria drugs are administered to malaria cases as a supplementary relief measure and are given routinely as a suppressive measure to workers on highway construction and other developmental projects in highly malarious areas. The drug of choice is chloroquine,[3] with some primaquine provided for radical cure treatments.

Although DDT residual spraying constitutes at least 95 per cent of the operations, other control measures such as automatic siphons, sanitoagronomic modifications and naturalistic methods are applied in a few appropriate situations. These more permanent measures are being utilized in developing the long-range program of malaria prevention and control, with primary emphasis being given to field demonstration projects in land development areas.

Plan of operations. The Six Year Program is being carried out by 30 Field Units and 25 Sub-units distributed throughout the malarious areas of the Philippines. The technical staff for these units have all completed a special course of training at the Malaria Field Center of the Project near Manila. The organization and equipment of the Units is generally similar to what has been used in many other DDT residual spraying programs, with of course adaptations to meet Philippine conditions. The plan of operations, however, incorporates some special features which may be of interest to others carrying out this type of program.

It was first estimated that about 1,350,000 houses would require spraying, but when the first nationwide survey was completed this was reduced to 1,050,000. The projected spraying schedule for these houses is as follows (houses given in thousands):

[1] Climax Junior No. 341, H. D. Hudson Mfg. Co., Chicago, Ill.
[2] "Tee Jet", Spraying Systems Co., Bellwood, Illinois.
[3] Chloroquine Diphosphate (Aralen) and Chloroquine sulfate (Nivaquine)

1952	1953	1954	1955	1956	1957	1958
5*	5*	5*	surveillance only			
	210	210	210	210	350	300
		835	835	835		
5	215	1050	1045	1045	350	300

* Mindoro Pilot Project.

It will be noted that following the third round of spraying now being applied (1954) to the five thousand houses in the Mindoro Project which were initially treated in 1952, spraying will be discontinued. The area will then be kept under careful surveillance during 1955 and 1956 to determine the effects of interrupted spraying upon the prevalence of malaria. Thus in 1957, when all houses will have received 3 or 4 years of consecutive spraying and the decision must be made as to what extent the nationwide spray coverage shall be reduced, there will be two years of data on interrupted spraying available from the Mindoro Project on which to base the decision. If the results indicate that it is desirable to extend complete nationwide coverage beyond the three-year period, facilities are available for continuing the operations at the present scope. Although the principle of interrupted spraying is believed to be sound and has been successfully applied in other countries (Livadas, 1953), the plan of operations for the Six-Year Philippine-American Program provides for flexibility in deciding when to apply this principle. In the meantime, it is estimated that by 1957 the nationwide spraying operations can be reduced by approximately two-thirds.

In the initial operations, houses are being sprayed in all meso-endemic areas (spleen rates above 10%) and in some hypoendemic areas where there is evidence of active malaria transmission. Complete coverage of all houses in an operational area is effected, although this may include scattered spots where there is no actual malaria transmission.

Appraisal. The first malaria map of the Philippines was provided by the nationwide rapid reconnaissance spleen surveys completed near the end of 1953. However, to provide for more accurate before-and-after data on the control operations, special index areas were established in each of the 30 Field Unit zones of operation. In these index areas detailed surveys were made before the first round of spraying, and are being repeated annually thereafter. These surveys include inspections for adult and larval mosquitoes, blood and spleen examinations, determination of infant parasite, morbidity, and mortality rates, and observations on the prevalence of other household insects and insect-borne diseases. The chief criterion of entomological effectiveness is the density of *A. minimus flavirostris* in sprayed houses, since it now appears that malaria transmitted by this species can be controlled without eradication of the vector. The most important index of the immediate effectiveness in preventing malaria transmission is the infant parasite rates, but adult parasite rates and spleen rates will assume increased significance in the latter part of the program.

The survey data from the index areas in the 30 operational zones make it

possible to compare effectiveness under the four types of climate which are prevalent in the Philippines and to determine if the results follow the same pattern as the Mindoro Project.

Accomplishments to date. During the first season (1953) of routine operations of the Philippine-American Malaria Control Program approximately 215,000 houses were sprayed and 85,000 persons were given medical treatment for malaria. It is estimated that these operations have saved between one and two thousand lives and have prevented over 300,000 cases of malaria.

At the beginning of the 1954 season all thirty Field Units and their Subunits were activated and the operations were expanded to a nationwide coverage. By the latter part of March, 1954, over half of all the houses in the malarious areas of the Philippines have been sprayed. There seems to be no doubt that the complete spraying of about one million houses will be accomplished by June of this year, bringing malaria protection to five million Filipinos.

It is estimated that the total cost of the Six-Year Program will be in the neighborhood of 2 million dollars and 14 million pesos. This is only a fraction of the economic losses which malaria brings to the Philippines in a single year.

We feel confident that for the first time in history we are on the road to bringing malaria under control in the Philippines. Such an achievement is indeed a fitting tribute to the friendship and the mutual assistance programs of our two countries.

SUMMARY

A brief review is given of the history and epidemiology of malaria in the Philippines, and of control practices up to the present day.

The Six-Year (1953–1958) Philippine-American Program for Malaria Control in the Philippines has for its objective the reduction of malaria to an insignificant level by annual spraying of all houses in malarious areas with DDT for at least 3 consecutive years, and thereafter spot-spraying of houses as local conditions demand. Concomitantly there is being developed a long-range program which consists of more permanent measures. Anti-malaria drugs are used as a supplementary relief measure. The total number of houses to be sprayed is about one million, with over four million house-sprayings to be carried out during the six year period.

During the first season's operations (1953) a total of 215,000 houses were sprayed and 85,000 people received treatment for malaria. During 1954 the program has been expanded to a nationwide spraying of one million houses, which will bring malaria protection to 5 million people.

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EFFECT OF BLOOD GROUP ON THE PREPATENT PERIOD OF INOCULATED *VIVAX* MALARIA

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Following the inoculation of plasmodia-infected blood for the treatment of central nervous system syphilis or for certain experimental studies on malaria, many workers have noted a marked difference in the incubation period (and in the prepatent period also) in recipients, even though the blood came from the same donor, was transferred at the same time, and each recipient received the same amount of inoculum.

The possibility that the compatibility of the blood between donor and recipient might be responsible for the differences in the length of the incubation period in blood-induced therapeutic malaria was first reported by Wendlberger (1927). He noted that the incubation period was prolonged if the donor's blood was incompatible with that of the recipient. In some recipients the malaria failed to develop at all, but when the inoculation was repeated with compatible blood malaria developed in most cases. Investigators who confirmed Wendlberger's findings, and their data pertinent to this discussion are presented in Table 1.

Other authors, including Pilcz (1927), Puntigam (1930), Walter (1929) and Arzt and Fuhs (1927), in reporting their experiences with blood-induced malaria for the treatment of syphilis stated that they noticed no definite correlation between the incubation period and the compatibility of the bloods.

In all of the above-mentioned studies there was no selection of patients as to racial or acquired immunity, and also there was no attempt to give a definite number of parasites. The usual practice was to inject 5 to 30 ml. of parasitized blood.

MATERIALS AND METHODS

In the course of our studies directed toward the evaluation of certain antimalarial drugs we were able to obtain data on the length of the prepatent period (i.e., the number of days from inoculation to the appearance of parasites in thick smears of peripheral blood) in 54 subjects inoculated with blood parasitized by the Chesson strain of *Plasmodium vivax*. Healthy adult white male volunteers from the inmate population of the U. S. Penitentiary, Atlanta, Georgia, none of whom had ever had malaria, were used as hosts. Each volunteer received approximately one million parasites intravenously; the amount of parasitized blood injected varied from 0.2 to 2.0 ml. Blood was transferred from a single donor on 13 separate occasions; the number of recipients at any one transfer ranged from one to sixteen.

RESULTS

The prepatent periods resulting from the inoculation of compatible and incompatible parasitized blood are shown in Table 2.

TABLE 1

Summary of studies showing compatibility of blood between donor and recipient as a factor in determining the length of the incubation period in blood-induced malaria

Author	Total No. of Cases	Incubation Period*	
		Compatible blood (days)	Incompatible blood (days)
Wendlberger (1927)	50	—	LTWCB
Wethmar (1927)	38	4.5	8.2
Hermann and Hlisnikowski (1928)	—	3.0	4.5
Engerth and Stumpfl (1928)	—	—	LTWCB
Hecht-Eleda (1928)	—	5.0	6 to 7
Hopf (1928)	—	—	LTWCB
Wagner von Jauregg (1929)	—	4.5	15.1
Wendlberger (1930)	—	4.5	10.7
Knights (1930)	12	6.4	9.8
Speierer (1930)	—	Not over 5	Up to 21
Bravetta (1931)	246	—	LTWCB
Klein (1931)	200	—	LTWCB
Ponisovskaya and Miniovitch (1931)	126	—	LTWCB
Muller (1931)	106	5.28	7.45
Somogyi and von Angyal (1933)	100	2.0	3.6
Polayes and Derby (1934)	127	4.3	8.18
Derby (1935)	287	4.4	7.68
Kranz (1942)	100	4.4	6.7

* All inoculations were made intravenously into syphilitic patients.
LTWCB = longer than with compatible blood.

TABLE 2

Prepatent periods in fifty-four volunteers who received intravenous inoculations of parasites in compatible and incompatible blood

Blood Group		Prepatent Period (days)
Donor	Recipient	
A	A (13)	2–5 mean 3.4
A	AB (2)	3,4
A	O (21)	8–22 mean 11.3
O	A (8)	3–5 mean 4.0
O	B (2)	4,4
O	O (8)	3–4 mean 3.8

Figures in parentheses denote number of recipients.

In only one series was the prepatent period increased significantly, and that was in group O recipients who had received blood from group A donors. The length of the prepatent periods in this group ranged from 8 to 22 days, with a mean of 11.3 days; however, it should be pointed out that among 16 of the 21 individuals, the range was 10 to 12 days. There were no reactions resulting from inoculation of incompatible blood in this group.

DISCUSSION

Although the prepatent period in blood induced malaria is directly proportional to the number of parasites injected, it is clearly evident that the compatibility of the blood between donor and recipient is also a very important factor. This has practical importance in at least two ways: (1) if a suppressive drug were under test, it might be assumed that the increased prepatent period resulted from the action of the drug rather than from the incompatibility of the blood; (2) if malaria were induced in a patient for therapeutic reasons, results might be more predictable if parasitized blood were obtained from a donor whose blood was compatible with that of the recipient. If incompatible blood must be used a much larger number of parasites should be injected if it is desired to produce malaria in the patient quickly.

SUMMARY

In 33 nonimmune subjects who received one million *Plasmodium vivax* parasites in blood compatible with that of the recipients the mean prepatent period was 3.7 days. In 21 nonimmune individuals of blood group O who received one million parasites of *P. vivax* in incompatible blood of group A donors the mean prepatent period was 11.3 days.

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THE ROLE OF *ENDAMOEBA HISTOLYTICA* AND *TRICHURIS TRICHIURA* IN BLOODY DIARRHEA OF CHILDREN IN PUERTO RICO

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Amebic dysentery is generally the diagnosis when increased frequency and fluidity of stools is associated with the passage of blood, mucus and actively motile trophozoites of *Endamoeba histolytica*. The failure of a small group of children exhibiting this syndrome to show uniform improvement when amebic infection was eradicated, and the observation that the signs, symptoms and therapeutic response correlated with the simultaneous overwhelming infection with *Trichuris trichiura* justify this communication.

METHOD

Sixteen children with bloody diarrhea whose stools showed the presence of both *E. histolytica* and *Trichuris trichiura* were selected for study. All gave a history of six to ten bloody stools a day for six months to two years. These children were admitted to three study groups chosen at random. One group received one gram of oxytetracycline daily for ten days as an agent of proven anti-amebic value (Martin *et al.*, 1953); the second group was given three enemata of 0.3% solution of Hexylresorcinol[3] in 10% acacia at 3 day intervals, a technic which has proven successful in markedly reducing total worm burden without demonstrable anti-amebic action (Jung and Beaver, 1951); while the third and control group received only supportive therapy and routine hospital care.

Proctoscopy was performed prior to and after completion of treatment. Stools of all children were examined daily for eggs, parasites and bacterial pathogens. Bedside isolation precautions were observed. The children were observed at least four days before therapy was started, and were followed for four weeks after therapy and then given appropriate additional medication prior to discharge.

RESULTS

No bacterial pathogens were isolated from any of the stools studied. Pretreatment proctoscopic examination in all cases revealed a friable hemorrhagic mucosa in which were imbedded myriads of adult *Trichuris trichiura*. Ulcers were infrequent. The bowel contents were fluid and all contained actively motile

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[3] Sharpe and Dohme Company and Laboratories Kuba generously supplied this material.

TABLE 1

Response of children with symptoms of "bloody diarrhea" to several treatment regimes

Results	Therapeutic Groups		
	Oxytetracycline	Hexylresorcinol	Supportive
Cleared of *E. histolytica*	6/6*	1/5	1/5
Cleared of *T. trichiura* eggs	1/6	2/5	0/5
Cleared rectally of *T. trichiura* adults	1/4	3/3	0/5
Improved consistency of stools	5/6	4/5	2/5
Cleared of melena	3/6	3/4	2/5
Decreased frequency of stools	6/6	5/5	2/5

* Number with result over number originally positive.

trophozoites of *E. histolytica*, many with ingested red blood corpuscles. Following therapy, few gross changes were observed in the oxytetracycline and control groups but the rectal lesions in the Hexylresorcinol treated groups showed uniform improvement. Microscopically, *E. histolytica* disappeared from all cases of the oxytetracycline treated group and from one case of the other groups.

At the completion of therapy (see Table 1), blood was still present in the stool of only one Hexylresorcinol treated patient; on the other hand, despite the disappearance of the *E. histolytica*, blood persisted in three oxytetracycline-treated patients; in the control group the blood cleared from two patients while *E. histolytica* persisted. Decrease in frequency of stools and diminution of their fluidity occurred despite the persistence of *E. histolytica* in four Hexylresorcinol treated cases.

DISCUSSION AND CONCLUSION

Interpretation of the etiologic factor of a specific sign or symptom is especially difficult in the presence of infection by multiple parasites. From this study it would appear that the presence or absence of *E. histolytica* need not be correlated with the fluidity and frequency of stools and associated passage of blood. It is known that following the administration of oxytetracycline in acute amebic colitis, bloody exudate ceases, amebae disappear and ulcers begin *epithelization* as early as 48 hours after institution of therapy (Martin *et al.*, 1953).

In this small series of cases, treatment with an anti-helminthic agent, ignoring the protozoa, appeared to have produced effective clinical end points, a finding similar to that reported by Jung and Beaver. Although the anti-amebic agent may have produced different collateral effects, the fact remains that symptoms persisted in several children despite the eradication of *E. histolytica*.

These results further support the view that study of dynamic interparasitic relationships holds the key to the elucidation of the pathogenesis of amebic infection. At any rate, in a given case, the observation of motile *E. histolytica* with ingested blood cells should not be the end of the study for the etiology of the patient's "bloody diarrhea".

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THE PREVALENCE OF *ENDAMOEBA HISTOLYTICA* AND OTHER INTESTINAL PARASITES IN A SELECTED URBAN AREA

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In previous reports we have described the prevalence of *Endamoeba histolytica* and other intestinal parasites in several rural population groups of Tennessee and Mississippi (Eyles *et al.*, 1953; Jones *et al.*, 1953a, 1953b). The present study extends the investigation to an urban area. It was made with the cooperation of the Memphis-Shelby County Board of Health and was conducted in a Negro and in a white housing project in urban Memphis, Tennessee.

The purpose in making this study was several-fold. In the first place, we desired to determine how the prevalence of parasites in city groups compared with prevalence in nearby rural areas. Secondly, there have been indications of higher prevalence of amebae in the white race in some of our previous studies, and we wanted to investigate the white and the Negro races under as nearly equivalent economic and social conditions as possible. Finally, we wished to correlate medical history with parasite findings in a population more enlightened than those we have previously investigated.

The housing projects studied are located near the center of Memphis. One is just adjacent to the business section and the other is less than two miles away. Both projects consist of modern brick buildings containing varying numbers of living units. The white development consists of 429 family units and the Negro of 721. The populations of the projects are 1496 and 2551 respectively.

Both projects are 14 years old. The tenants have been resident for varying periods of time, the average being 2.8 years for the white families studied and 1.3 years for the Negro. Most of the tenants came into the projects from an environment which was poorer from a sanitary standpoint. Many came from substandard residences.

The economic status of the tenants of the two projects varied slightly. White families had an income average for all members of $1,920 per annum compared with $1,560 for the Negro. In the case of the white project the average family income was below that of the general city white population; whereas, in the case of the Negro families the mean income was about the average for the City (U. S. Bureau of the Census, 1952).

METHODS

Complete rosters of the housing projects were obtained, and families were circularized at random to determine which would be willing to cooperate in the

[1] Located at the Medical School of the University of Tennessee, 874 Union Avenue, Memphis 3, Tennessee.

study. Response to this circularization was poor, and as a consequence it was necessary, particularly in the Negro project, to solicit cooperation from door to door. The final samples consisted of about 18 per cent of the total population of the projects. It must be recognized that the sample is biased in that the health-conscious families were most likely to cooperate.

After families agreed to participate in the study, the following basic data were obtained: 1. roster of family members with age and sex; 2. duration of tenancy in project; 3. occupation of head of household. In addition data on the medical history of the family were solicited, especially with regard to the occurrence of diarrhea, chronic constipation and abdominal pain.

Following the securing of the above data, three normal stool specimens were collected at weekly intervals. These were examined directly in iodine and after zinc sulfate concentration as in our previous studies (Eyles *et al.*, 1953). Most of the diagnoses were based upon the identification of cysts of the various protozoa. In addition, many specimens were cultured in Nelson's medium (Nelson, 1947) and examined after 48 to 72 hours for amebae.

RESULTS

The overall prevalence rates by individuals from the two projects are summarized in Table 1. A direct comparison can be made between the two projects, and the present results can be compared also with the results of our previous rural studies, since the technical procedures were identical. This tabulation does not include data from the culture studies.

E. histolytica was more prevalent in the white population than in the Negro. Similarly, *Endolimax nana* showed a higher white race prevalence; but *Endamoeba coli* was slightly more prevalent in the Negro group. Chi-square test showed that none of these differences were statistically significant according to the usual standards ($p < .05$); however, the probability that the observations in the case of *E. histolytica* were due to chance was only 0.10.

Intestinal amebae were most prevalent in the 15- to 19-year age group as is shown in Figure 1. The female sex was parasitized to a slightly greater degree by *E. histolytica* and *E. nana*, but there was no difference in the occurrence of *E.*

TABLE 1

Prevalence rates of intestinal protozoa in two Memphis housing projects

	White Project			Negro Project			Both Projects		
	No. exam.	No. pos.	Per cent positive	No. exam.	No. pos.	Per cent positive	No. exam.	No. pos.	Per cent positive
Individual prevalence									
E. histolytica	336	16	4.8	397	10	2.5	733	26	3.5
E. coli	336	43	12.8	397	59	14.9	733	102	13.9
E. nana	336	47	14.0	397	40	10.8	733	87	11.9
I. bütschlii	336	4	1.2	397	4	1.0	733	8	1.1
G. lamblia	336	18	5.4	397	19	4.8	733	37	5.0
C. mesnili	336	0	0.0	397	5	1.3	733	5	0.7

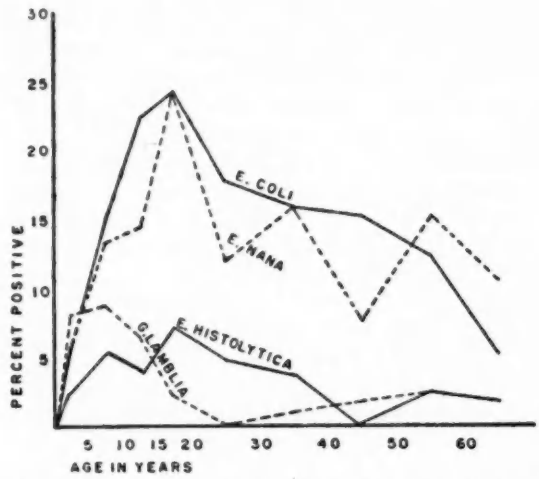

FIG. 1. Graph showing prevalence rates of intestinal protozoa by age of individuals in two Memphis housing projects.

coli in the two sexes. *Giardia lamblia* was most prevalent in the group under five years of age, and slightly more males than females were found parasitized. In general, the prevalences by age and sex of host followed the pattern observed in other studies in the mid-south area (Eyles *et al.*, 1953; Jones *et al.*, 1953), in that the female sex tended to have more amebic infections and highest prevalences were in pre-adult age groups.

Prevalence rates for the intestinal protozoa in these urban populations were markedly lower than those found in rural communities in the same area (Table 2). The amebae occurred about one-third as often with the exception of *E. coli* which was found a little more than half as often in the city. *G. lamblia* was less than half as prevalent in the city groups.

The proportionate prevalence of the different amebae in the city and rural area was similar, except that rates for *E. nana* were much higher than those for *E. coli* in the rural communities; whereas, *E. coli* was slightly more prevalent in the urban area.

Helminths, with the exception of *Enterobius vermicularis*, occurred infrequently in these housing projects. Investigation disclosed that four white individuals harboring *Necator americanus* and one with *Ascaris lumbricoides* had ample opportunity to acquire these parasites in poorly sanitated rural sections adjacent to Memphis. In addition, two individuals in the Negro project were found to harbor *Hymenolepis nana*.

During discussions with the mothers, it became apparent that *E. vermicularis* was very prevalent, especially in the white population. The limitations of our study prevented the special examination necessary to disclose the percentage prevalence of this parasite.

TABLE 2

Comparison of prevalence rates of the important intestinal protozoa in urban and rural populations

	Per Cent Positive with Standard Error		
	Urban	Rural	
	Memphis housing projects (733 persons)	Fayette County Tenn. (2,657 persons)	Yazoo-Miss. Delta (935 persons)
Endamoeba histolytica	3.5 ± 0.7	10.5 ± 0.6	10.8 ± 1.0
Endamoeba coli	13.9 ± 1.3	22.2 ± 0.8	19.0 ± 1.3
Endolimax nana	11.9 ± 1.2	29.1 ± 0.9	30.2 ± 1.5
Giardia lamblia	5.0 ± 0.8	9.0 ± 0.6	12.8 ± 1.1

Medical history and parasite findings. At the time each family was interviewed, simple questions were asked, usually of the mother, as follows: 1. Had any individual in the family suffered from diarrhea (or dysentery) during the previous 12 months? 2. Was any individual troubled with chronic constipation? 3. Did any individual suffer from recurrent abdominal pain, particularly in the right upper quadrant? All data were obtained before parasitological examination was made. The data were analyzed to determine whether or not the findings were associated with the occurrence of *E. histolytica* or the other intestinal amebae.

Considering first the possible association of diarrhea and intestinal amebic infection, simple four-fold chi-square tables were constructed as in Table 3. It can be seen that, while *E. coli* and *E. nana* showed no significant degree of association, *E. histolytica* and diarrhea occurred together more frequently than could be accounted for by chance association alone. Nearly one-third of the persons with *E. histolytica* reported diarrhea, whereas only about one out of seven persons of the overall group reported this symptom.

In addition to the direct and concentration examination performed on specimens from the population for this study, many of the specimens were cultured as part of another project (an unpublished study of the efficiency of different techniques). Believing that the inclusion of the results from the culture studies might give a more nearly true picture of the real prevalence of *E. histolytica*, the analysis to determine how frequently diarrhea and the parasite were associated was repeated using these data. This analysis is also presented in Table 3, and it can be seen that the use of these data does not change the conclusion; in fact, the statistical probability of the finding being due to chance is more remote in this case.

There was no correlation between the occurrence of any parasite and chronic constipation or abdominal pain.

Relationship of duration of residence in the projects to prevalence of amebae. It was previously pointed out that many of the families in the housing projects had come originally from less sanitary environments. An analysis of prevalence rates based on the duration of residence was made to determine the effect of the

TABLE 3

Correlation between amebic findings and medical history in Memphis housing projects

	Diarrhea Reported	Diarrhea Denied	Chi-Square and Probability
Patients with *E. histolytica*	8	18	$X^2 \pm 4.93$
Patients without *E. histolytica*	95	612	$P = <.05$
Patients with *E. coli*	16	86	$X^2 = 0.13$
Patients without *E. coli*	87	544	$P = >.70$
Patients with *E. nana*	10	77	$X^2 = 0.88$
Patients without *E. nana*	93	553	$P = >.30$
Patients with *E. histolytica* (including culture data)	16	40	$X^2 = 9.32$
Patients without *E. histolytica*	87	590	$P = <.01$

previous environment on the present prevalence rates. There were no material differences between prevalences related to length of residence.

DISCUSSION

The present report, dealing with a selected urban group, provides data for comparison with findings of studies in nearby rural areas in which identical parasitologic and epidemiologic protocols were followed.

This limited study indicates that amebic parasites are much less frequent in the city than in the rural areas of this section. Results from the housing projects cannot be taken to represent the City of Memphis as a whole; however, they are representative of an urban group with modern sanitary facilities, living under conditions which are probably little better than average for the entire city.

Approximately the same relative trends in prevalence for age groups were observed in the city and in the rural communities; that is, parasites were more frequently found in the pre-adult groups. This may be taken as an indication that in the city, as in the country, transmission occurs mainly in the home rather than through the medium of food handlers in public eating places. If the latter were the case, major prevalence would be expected in adults who have more extra-home contacts.

Although the trends were similar, peak prevalence was noted in an older age group of individuals in the city than in the rural areas. This seems to be a further indication that exposure to infection is more frequent under rural living conditions than in the city and that infections are acquired at an earlier age under these conditions.

In the city we observed virtually the same distribution of parasitism in the sexes as that found in rural areas. The slight tendency toward higher infection rates in the female groups is not in accord with some reports from urban areas (Svennson, 1935). This finding is not in keeping with the opinion held by some that the fact that males customarily eat more meals away from home indicates

that they are more exposed to risk of infection than are the home-dwelling females. It should be added, however, that in the housing project population, many of the adult females were employed and presumably dined away from home.

Regarding the question of the association of amebic prevalence and race we are left in doubt. Although the usual standard of significance was not met, in the case of *E. histolytica* the probability of the observed racial difference in prevalence being due to chance was only 0.10. Certainly this justifies further study. This is especially true in view of the fact that some factors were noted which would ordinarily predispose toward greater prevalence in the Negro group as for instance larger Negro family size and lower sanitary standards.

A novel observation was that whereas, in the nearby rural areas *E. nana* was much more prevalent than *E. coli*, in the city *E. coli* was slightly more prevalent than *E. nana*. This recalls the study of Spector and Hardy (1939) in New Mexico where *E. coli* was found to be much more prevalent than *E. nana*. In the present case, the same laboratory technicians made both sets of observations; therefore, we can conclude that we were observing a real difference in the relative prevalence rates. It is possible that this indicates a difference in the life cycles of *E. nana* and *E. coli*.

The correlation between the occurrence of diarrhea and *E. histolytica* which resulted from the analysis of data from the medical histories was unexpected. Medical histories obtained by questioning, as in this study, are on the whole unsatisfactory. It is difficult, for instance, to classify the individual who reports a loose stool on one occasion but recalls no frank symptoms of diarrhea during the past year. However, every effort was made to obtain objective answers to the questions and to make an unbiased classification of this information before considering the parasitic findings in each case.

It is our opinion that the best explanation for the significant degree of association between *E. histolytica* and diarrheal symptoms is that this parasite caused the symptoms in many of the subjects investigated. This is supported by the absence of such association in the case of the intestinal amebae commonly believed to be nonpathogenic. We have been unable to form any other logical hypothesis to explain the findings. The study shows that there are many carriers of *E. histolytica* who reported no symptoms; however, the large proportion of the population with this parasite would make it a major producer of disease if the ratios measured by us are representative of the overall population.

SUMMARY

A study of *Endamoeba histolytica* and other intestinal parasites was conducted in two very similar urban housing projects, one inhabited by Negro and the other by white families, with the following results:

1. Observed prevalences of the intestinal parasites for this urban population were: *E. histolytica*, 3.5 per cent; *Endamoeba coli*, 13.9 per cent; *Endolimax nana*, 11.9 per cent; *Iodamoeba bütschlii*, 1.1 per cent; *Giardia lamblia*, 5.0 per cent; and *Chilomastix mesnili*, 0.7 per cent.

2. The higher prevalence rates for *E. histolytica* and *E. nana* and the lower

rate for *E. coli* in the white population were not significant statistically; however, other factors operating in the two populations which suggest the possibility of real racial differences are discussed.

3. Overall prevalence rates were much lower than those found in rural communities of the mid-south but the sex and age distributional patterns were, in general, the same.

4. The helminths occurred infrequently probably because of the excellent sanitary facilities.

5. The occurrence of diarrhea and *E. histolytica* infection were associated to a marked degree, but no further association between symptoms and parasites could be established.

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THE RELATION OF THE NUMBER OF CYSTS IN THE STOOL INOCULUM TO THE INCIDENCE OF POSITIVE CULTURES OF *ENDAMOEBA HISTOLYTICA*

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In previous reports, it was shown that the addition of small amounts of streptomycin and penicillin to culture media favored the cultivation of trophozoites of *Endamoeba histolytica* from cysts present in stool specimens (Spingarn and Edelman, 1948; 1952). Although the use of the antibiotics produced a substantial increase in the number of positive cultures obtained, excystation and multiplication of trophozoites was not observed in about one-third of the trials. A consideration of the possible factors involved in the negative cultures suggested that in these specimens the number of *E. histolytica* cysts was insufficient for cultivation or that the nature and quantity of the bacterial and fungal associates present prevented it. It is the purpose of this communication to report the results of a study of the relation of the number of cysts in the inoculum to the cultivation of trophozoites of *E. histolytica.*

METHODS

Stool specimens containing cysts of *E. histolytica* were obtained from 27 patients with amebiasis at the Mount Sinai Hospital. The cysts were identified microscopically and the specimens were cultured after 24 hours of refrigeration by a method described in our previous reports (Spingarn and Edelman, 1948; 1952). Specimens in which trophozoites as well as cysts were found were not included in this series. In addition, stools obtained from 50 other patients in which no cysts or trophozoites were found after study of direct smears and concentrates obtained by zinc sulfate flotation, were cultured in a similar manner.

Prior to inoculation, the numbers of cysts present in the positive specimens were estimated by the following method. Saline smears were prepared in a routine fashion on 1 x 3 inch glass slides. A small amount of feces was thoroughly emulsified in a small drop of normal saline on the slide and was then covered by a 22 mm. cover glass. The emulsion was of a density through which small newsprint could be read without difficulty. All portions of the cover slips were then examined microscopically and a count was made of the cysts present. Four smears were examined from different parts of each stool and the average of the cyst counts was used to denote the number present. For comparison, cysts were counted after concentration of material by zinc sulfate flotation from the same specimens. Although larger numbers of cysts were obtained and the values were roughly proportional to the direct counts, a considerable variation in the results from specimen to specimen led us to discard this method for quantitating the number of cysts present. On the other hand the cyst counts from smears of an emulsion of one gram of stool and 5 cc. of sterile normal saline solution, used as

the inoculum in the method of cultivation described below, agreed closely with the direct smear counts.

Each of six culture tubes containing a slant of *Endamoeba* medium (Difco) and a serum-saline overlay was inoculated with 0.1 cc. of inoculum from the emulsions. Two of the tubes contained 3 and 6 mg. of streptomycin sulfate respectively, two contained 3,000 and 6,000 units of crystalline potassium penicillin respectively, and two untreated tubes were included as controls. As in our previous work, the drugs were added to the serum-saline overlay in each tube. The tubes were incubated at 37°C. and were examined daily for amebae for seven days.

RESULTS

From the 27 stool specimens as shown in Table 1, positive cultures of trophozoites were obtained in 17 (63%). In the group of 50 stool specimens negative by microscopical examination for *E. histolytica*, only one positive culture of trophozoites was obtained. The incidence of positive cultures increased directly with the numbers of cysts in the inocula as shown in Tables 1 and 2. Among 7 cultures whose inocula contained 5 cysts or less per cover slip, only one was positive; of 9 cultures whose inocula contained between 6 and 12 cysts, 5 were positive; and in the last group of 11 cultures whose inocula contained between 15 and 300 cysts, all were positive.

The superiority of the antibiotic treated tubes over the untreated controls was demonstrated by the occurrence of growth in only 4 groups of controls (15%), as shown in Table 2. Moreover, trophozoites appeared more frequently in the antibiotic tubes inoculated with small numbers of cysts than in the control tubes. In no instance was a positive culture obtained in the controls when the cyst count

TABLE 1

Relation of number of cysts per cover slip to results of ameba cultivation

Number of Cysts per Cover Slip	Number of Stool Specimens	Results of Culture	
		Positive	Negative
1	4	0	4
2	1	0	1
3	1	1	0
5	1	0	1
6	3	2	1
10	5	3	2
12	1	0	1
15	2	2	0
35	1	1	0
40	2	2	0
100	4	4	0
150	1	1	0
300	1	1	0
Total	27	17	10

TABLE 2

The effect of antibiotic treated media on the cultivation of* E. Histolytica *from Stool Specimens*

Number of Cysts Present per Cover Slip	Number of Stool Specimens	Number of Successful Ameba Cultures	
		Without antibiotics	With antibiotics
1–5	7	0	1
6–12	9	1	5
15–300	11	3	11
Total	27	4	17

* Penicillin or streptomycin.

was less than 10, and in cultures inoculated with counts of from 10 to 100 cysts, only one control yielded trophozoites, whereas 8 positives were obtained in tubes treated with antibiotics. Among 108 culture tubes treated with antibiotics, 44 were positive (41%) in contrast to 6 positive (11%) among 54 controls. There was no difference in the number of positive cultures obtained with penicillin or streptomycin. It was also noted that the initial appearance of trophozoites did not occur after the fourth day following inoculation in the controls, whereas in the antibiotic treated cultures, in 7 instances trophozoites were seen for the first time on the fifth or sixth day.

COMMENT

The above data indicate that the number of cysts present is an important factor determining the success of cultivation of trophozoites of *E. histolytica* from stool specimens. With a large number of cysts (15 or more per cover slip), positive cultures frequently occurred with media treated with streptomycin or penicillin in contrast to the small number of positive controls. With smaller numbers of cysts (less than 15) positive cultures were obtained in only 37% of the antibiotic treated tubes and in only one of the controls (11%). This is a rough quantitative demonstration of the superiority of media containing streptomycin and penicillin over the untreated media. As indicated in our previous reports, the reduction in the total bacterial population produced by streptomycin and penicillin seemed to facilitate amebic growth in vitro (Spingarn and Edelman, 1948; 1952). It appeared that antibiotics retarded the rapid destruction of the nutrient properties of the medium by bacterial overgrowth and the disintegration of rice starch, an important stimulant of amebic growth. As shown in the present study, the suppression of the associated flora reduced the number of cysts required for positive cultivation.

It has been stated that cultivation is of value in the detection of *E. histolytica* in stool specimens when microscopic examination of fecal smears is unsuccessful. (St. John, 1926; Tsuchija, 1942; Michael and Cooray, 1952; Anastopoulos, 1949; Lucas, 1945; Belding, 1952; Laird *et al.*, 1949). According to our data, the likelihood of obtaining a positive ameba culture was small when the stool specimen contained only a few cysts per cover slip on direct examination

in spite of the addition of streptomycin or penicillin to enhance the sensitivity of the medium. The high percentage recoveries of *E. histolytica* in culture compared to direct microscopic examination reported by others may have been due to differences of technique, since our work was done with a single medium and care was taken to exclude stool specimens containing trophozoites as well as cysts. Although we agree with Laird and his co-workers (1949) that a reliable cultivation method would be desirable as a routine procedure in the diagnosis of amebic infection, in our hands microscopic examination of fecal smears has continued to be the most dependable method for the routine diagnosis of *E. histolytica* in the laboratory.

SUMMARY

A direct relationship was noted between the number of cysts of *E. histolytica* present in stool specimens and the incidence of positive cultures of trophozoites. Positive cultures were invariably obtained when 15 or more cysts per cover slip were counted by direct microscopic examination. The addition of streptomycin or penicillin to the culture medium made it more favorable for the cultivation of specimens containing a small number of cysts. One positive ameba culture was obtained from fifty specimens microscopically negative for cysts of *E. histolytica*.

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A COMPARATIVE STUDY OF FUMAGILLIN AND OXYTETRACYCLINE IN AMEBIASIS

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Among the currently available antibiotics used in the treatment of amebiasis only fumagillin is believed to act solely as a direct amebicide (McCowen *et al.*, 1951). The other antibiotics appear to act principally on bacterial associates necessary to survival and growth of amebae and are thus indirectly effective in the elimination of amebae (Anderson and Anderson, 1950).

In vitro testing has demonstrated that fumagillin is one of the most effective of the amebicidal agents available, being active in dilutions as high as 1:130,000,000 (McCowen *et al.*, 1951). On the basis of this information and the data from satisfactory toxicologic studies in animals, trials in man were initiated at this institution.

In 1952 a preliminary note on the experiences with fumagillin in 22 patients with amebiasis was published (Killough *et al.*, 1952). The largest dosage administered to a patient in that series was 50 mg. daily for 15 days. One patient with dysentery did not respond to treatment with 50 mg. daily for 14 days and three patients who received 5 or 10 mg. daily for 14 days again showed *Endamoeba histolytica* in stools while still hospitalized. The remaining 18 patients were negative during the follow-up period of two months. Subsequently, the dosage has been increased and this series has been enlarged to a total of 67 patients, some of whom have been followed for two years. The present report is on the completed series. For comparative purposes, the data on a group of 48 patients treated with oxytetracycline (Terramycin) and followed for a similar period are reported.

METHODS

Sixty-seven male patients were treated with fumagillin for microscopically proved *Endamoeba histolytica* infection. The patients ranged in age from 14 to 59 years, averaging 28. All were of the poorest class, coming from highly unsanitary environments. Multiple parasitic infections were present in all. In the entire series, 14 species of parasites were identified (7 of protozoa, 2 each of blood flukes and round worms, and 1 each of hookworm, dwarf tapeworm and pinworm).

During fumagillin therapy, stool examinations were performed daily using direct saline smear and the MIF technic (Sapero *et al.*, 1951). Thereafter, exami-

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[2] This study has made possible through the cooperation of the Egyptian Ministry of Health.

[3] The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

TABLE 1

Recurrences of Endamoeba histolytica *in relation to dosage of fumagillin and duration of follow-up*

Dosage mgm/d	Number of patients	Recurrence in months post-treatment					
		In hospital 0–½	Outside hospital				Total
			1½–4	4–8	8–12	12–18	
5–50	22	5	5	5	0	0	15
51–100	20	0	3	0	0	1	4
101–150	15	1*	1	2	1	0	5
151–200	10	1*	1	4	1	1	8
	67	7	10	11	2	2	32

* Patients with dysentery.

nations were performed one to three times weekly until the termination of hospitalization. Other laboratory studies before, during, and after treatment consisted of complete blood counts, frequent urine examinations, stool and urine cultures when indicated, blood urea nitrogen, urea clearances, electrocardiography and a group of eight liver function tests.

All patients receiving fumagillin were treated for 14 days. The total daily dosage ranged from 5 mg. to 200 mg. and was divided into two or three portions for administration. For convenience in discussion, the patients are divided on the basis of dosage into four groups, (Table 1). Twenty-two patients received 5 to 50 mg./d; 20 received 51 to 100 mg./d; 15 received 101 to 150 mg./d; and 10 received 151 to 200 mg./d.

A similar group of 48 male patients was treated with oxytetracycline. Thirty-two of these were admitted primarily for the treatment of *Brucella melitensis* infections but had concomitant infections with *Endamoeba histolytica*. The remaining 16 patients had amebic dysentery. With the exception of renal clearances, the same laboratory procedures were performed on these patients as on those receiving fumagillin.

The dosage of oxytetracycline varied considerably from one group of patients to another but in none was the dosage less than that recommended as effective by other investigators (Frye *et al.*, 1952; Martin *et al.*, 1953). Of the patients with brucellosis, two received 2 grams daily for 10 days; 11 received at least 2 grams daily for 2 weeks; 16 received 2 grams daily for 3 weeks and 5 received 2 grams daily for 6 weeks. All of the patients with dysentery were treated for 14 days, 10 receiving 4 grams daily and 6, 2 grams daily.

The follow-up for both groups of patients was continued for a minimal period of four weeks in the hospital. After discharge, patients were recalled for interval histories, physical examination and stool studies. The periods of follow-up for the fumagillin and Terramycin treated groups have ranged respectively from 15 to 24 months and 12 to 25 months. The average number of return visits and stool examinations during the follow-up has been 19 for each of the fumagillin and 8 for each of the Terramycin-treated patients.

RESULTS

A. *Patients with dysentery*

(1) *Fumagillin treatment.* The 13 patients with dysentery are considered separately because of the striking difference in severity of illness, response to treatment and recurrence of infection. Eleven had stools that were grossly bloody, and five had rectal temperatures exceeding 100°F. In each there was tenderness over the area of the large intestine. The liver was palpable and tender in all except two patients and, in these two, although the liver was not palpable, there was tenderness to fist percussion over the hepatic area.

The symptomatic response to treatment with fumagillin (50–200 mg./d) varied widely but not in relation to dosage. In 11 patients, the initial symptoms disappeared between the ninth and fourteenth days of treatment. However, in two patients the dysenteric symptoms were not controlled and both developed signs of hepatic abscess. After 14 days of fumagillin (50 mg./d) one was changed to oxytetracycline and chloroquine. This patient recovered slowly becoming afebrile after 10 days. The other patient, who had had fever and bloody diarrhea for one month before admission, died on the tenth day of hospitalization having received fumagillin (120 mg./d) for 10 days and concomitant chloroquine for 8 days. Stools had become negative for amebae on the third day of treatment and had remained negative in thirteen subsequent examinations. At autopsy a large unruptured hepatic abscess and extensive colonic ulcerations were found. Amebae were not recovered from the lesions.

Proctoscopic examination on admission on the patients with a dysentery revealed extensive ulcerations. The lesions were of such severity as to preclude complete proctoscopy due to the danger of possible perforation. In the patient who died, the ulcerated area was far greater than that of the intervening mucosa. With the exception of this patient, ulcers had decreased in size by the fifth or sixth day of therapy. In one patient with relatively small ulcerations, healing was complete by the tenth day of treatment, but in others the healing was not completed until the week after completion of therapy. Roughly, the time required for healing seemed to be inverse to the area of ulceration and without relation to dosage.

The parasitic response was rapid in all except one patient. Within 72 hours, the specimens from 12 were negative for amebae. In the remaining patient, however, the stools did not become negative until the eighth day and were again positive within 24 hours of the last dose of fumagillin.

Of the 12 surviving patients, seven had a recurrence of amebae within one day to six months after termination of fumagillin. In two, this occurred while still hospitalized where the likelihood of reinfection is low.

(2) *Oxytetracycline treatment.* Sixteen patients were treated for dysentery with oxytetracycline. On admission 15 had stools that were grossly bloody and six had fever. All had tenderness over the liver and large intestine. In 12 patients, symptomatic response to treatment occurred between the second and fourteenth day. One patient continued to have abdominal cramping until one week after the termination of treatment. Three patients had amebic abscess of the

liver. One had an unrecognized hepatic abscess on admission and although he stated that he was symptomatically improved, he continued to be febrile and stools contained trophozoites. Suddenly on the sixth day of treatment he lapsed into coma and died. Another patient responded satisfactorily both clinically and parasitically but on the ninth day post-treatment developed fever and a recurrence of hepatic tenderness and dysentery with trophozoites. He did not respond to oxytetracycline and chloroquine and died on the eighth day of treatment with dysentery of such severity that *Wuchereria bancrofti* were identified in stool specimens along with numerous amebae. At autopsy, both of these patients had extensive large bowel ulcerations and hepatic abscesses. In the second patient *Endamoeba histolytica* was cultured from a hepatic abscess in spite of eight days of chloroquine therapy. A third patient developed fever and signs of hepatic abscess on the eleventh day of treatment but had a rapid response when chloroquine was added to the antibiotic.

Twelve of the patients with dysentery were proctoscoped more than once. All had extensive ulceration on admission. Improvement in the appearance of the bowel was apparent during the first week of therapy but the time required for healing varied from the sixth day of treatment to 18 days after the termination of therapy.

Eight of the 14 surviving patients have had recurrence of amebic infection but without a return of symptoms. In one of these patients the amebae reappeared seven days after completion of therapy.

B. *Patients without dysentery*

(1) *Fumagillin treatment.* Among the 54 patients with nondysenteric amebiasis who were treated with fumagillin, 17 were asymptomatic and without significant physical findings. The remaining 37 patients complained in varying degree of abdominal colic, tenesmus, epigastric pain and occasional attacks of diarrhea. On physical examination tenderness over a segment of the colon and a minimal degree of hepatic tenderness were most common.

Stools were negative for amebae within 72 hours of the initiation of fumagillin in all except three patients. In these, stools were negative at 96 hours. During the first week of treatment, complaints of colic, tenesmus and epigastric pain disappeared. Hepatic and colonic tenderness disappeared between the third and nineteenth days.

The distribution of recurrences in all patients treated with fumagillin is pre-

TABLE 2

Recurrences of Endamoeba histolytica *in patients treated with fumagillin and patients treated with oxytetracycline*

Drug	Number of patients	Months post-treatment					Per cent with recurrence
		0-4	4-8	8-12	12-18	18-	
Fumagillin	45	7	6	2	2	0	37.8
Oxytetracycline	48	2	4	9	2	5	45.8

sented in relation to daily dosage and duration of follow-up in Table 1. The over-all incidence of relapse or reinfection including the patients with dysentery, is 48 per cent. Eliminating the patients with dysentery, however, does not significantly alter the results (46%). Excluding those patients receiving less than 50 mg. daily who are considered to have received inadequate dosage, the recurrence rate is lowered to 38 per cent (Table 2).

(2) *Oxytetracycline treatment.* The response of 32 nondysenteric patients treated with oxytetracycline was essentially the same as that of the group receiving fumagillin. Recurrence of amebae during follow-up was also similar (Table 2). The over-all incidence is 46 per cent and the elimination of patients with dysentery does not significantly alter the results (44%).

C. *Side-effects from fumagillin*

Data on side effects of fumagillin are presented in Table 3. The most significant in terms of distress to the patient was anorexia, appearing on the second or third day of treatment. Although severity roughly paralleled dosage, there was considerable individual variation. Even at 200 mg. daily there was one patient who gained in weight and had no complaint. On the average, however, the patients in all groups lost weight during treatment, the range being from one to seven pounds.

Sixteen patients receiving more than 50 mg. daily complained of nausea and seven of these vomited occasionally. As with anorexia, the nausea and vomiting appeared on the second or third day of fumagillin. None developed diarrhea. All symptoms referable to the gastro-intestinal tract disappeared within 24 to 48 hours after termination of treatment. Following treatment all patients gained weight rapidly so that on discharge none weighed less than on admission.

Dermatitis was a complaint of approximately one quarter of the patients. The distribution varied considerably. Lesions appeared on the face, neck, hands, feet, chest and abdomen, but no single patient had lesions in all of these areas. Between the fourth and twelfth day of medication there would appear on the face faintly erythematous areas accompanied by slight burning and occasionally pruritus. Distribution was symmetrical and always covered large areas, at times the entire face and neck area. Simultaneously or sometimes preceding the burning there would appear fine white scaling. Lesions did not progress beyond this stage and within one week post-treatment, clearing was complete. Vesiculation was not observed. At approximately the same time as the facial lesions, there might appear a fine papular eruption on the dorsal and interdigital surfaces

TABLE 3

Toxic reactions in relation to daily dosage of fumagillin

	5–50 mgm.	51–100 mgm.	101–150 mgm.	151–200 mgm.
Anorexia	3 (14%)	13 (64%)	12 (80%)	9 (90%)
Weight loss	11 (50%)	17 (85%)	13 (87%)	9 (90%)
Dermatitis	1 (5%)	5 (25%)	4 (27%)	2 (20%)

of the fingers. This would progress in one or two days into fine vesicles containing clear serous fluid. These gradually dried and within one week were succeeded by coarse flaking of the superficial layers of the skin with frequent extension into the palm. In none of the patients was an area denuded of epithelium and the rash always subsided without special care. Lesions similar to those on the hands frequently appeared on the feet and in one patient also on the abdomen and chest. One patient complained of burning and perianal pruritus and two patients receiving less than 50 mg. daily complained of transient dizziness. In no patient was it necessary to discontinue fumagillin because of these side-effects.

DISCUSSION

As stated by Sodeman and Beaver (1952): "When amebas are discovered in the post-treatment stools of non-hospitalized individuals, there is justifiable doubt about the origin of the infection." This is particularly true in an overpopulated and unsanitary area where patients return on discharge to the heavily endemic environment from which the infection was acquired. The patients described in the present study returned to villages where on single stool examination as many as 47 of 88 individuals have been found to harbor *Endamoeba histolytica*, and where 97 per cent of 80 individuals have been found to have positive stools at some time during a two and one half year study (Lawless, 1953). It is for this reason that the term recurrence is used instead of relapse in most of this report.

For the same reason it is felt that little significance can be attached to prolonged follow-up data such as that presented in Table 2. These recurrences may as well be a measure of the rate at which nonparasitized individuals acquire amebae as actual relapses. It is interesting to note that there is no significant difference in the number of positives found in the two treatment series during prolongation of follow-up. This suggests that fumagillin and oxytetracycline are of equal effectiveness. However, the actual cure rate of these drugs cannot be determined under circumstances where one requires a patient to be studied for 6 months to a year or more to establish cure.

It is felt that this requirement of prolonged follow-up is currently fulfilled in an unsatisfactory manner. Investigators generally lack data as to the rate at which infection is acquired in an area and they also lack data on spontaneous cure. Studies have been made on these questions in certain areas, but as yet the data include relatively small numbers (Frye *et al.*, 1952). One of these complications can be eliminated by follow-up in the controlled hospital environment. Sodeman and Beaver (1952) have used this procedure and it has been used in the present investigation. How long patients should be followed to establish a cure as definite can be answered in such studies. Of the four patients receiving fumagillin and one receiving oxytetracycline who were followed during hospitalization in this study for over 6 months, none had a recurrence of amebae. Without more of such information one cannot be dogmatic on the subject, but it is felt that 4 to 8 weeks study after treatment in a hospital environment is more reliable than 6 months or more in a less clean environment. If such is true, both fumagillin

and oxytetracycline are very effective in the treatment of nondysenteric amebiasis.

The necessity for considering severity of disease when evaluating the effectiveness of curative therapy is apparent from the present study. The only recurrence of parasites among hospitalized patients followed treatment for dysentery. All nondysenteric patients remained free of amebae following oxytetracycline and adequate dosage of fumagillin (more than 50 mg. daily) to the end of at least 4 weeks hospitalization. For this reason, patients with frank amebic dysentery have been presented separately from patients with asymptomatic or mildly symptomatic amebiasis. A similar observation was made by Manson-Bahr and Muggleton (1948) when they found that diodoquin was effective in curing infections in which only cysts were passed but valueless in dysentery.

Parasitic response to fumagillin in patients with dysentery was similar to that of the group treated with oxytetracycline. Symptomatic response also was approximately the same, symptoms disappearing in 9 to 14 days in patients receiving fumagillin as compared with 2 to 14 in those given oxytetracycline. Fever, abdominal colic, tenderness and bloody diarrhea disappeared approximately at the same time. Throughout the study, there was an impression that the healing of ulcerations in the fumagillin-treated was slower but this cannot be supported from the data since there is no way adequately to objectively quantitate the extent of lesions either before or during therapy. The presence of secondary bacterial invaders uncontrolled by fumagillin, however, logically would suggest an advantage of oxytetracycline in facilitating the healing of ulcers.

Neither of these antibiotics has demonstrated effectiveness in the management of amebic abscess. Both with fumagillin and with oxytetracycline patients having dysentery developed abscesses while under active therapy, and in both death has occurred in severely ill patients with prolonged dysentery and abscess. This has occurred even when chloroquine was used in conjunction with the antibiotic.

Also actual eradication of the *Endamoeba histolytica* infection at the time dysentery is treated has been unsatisfactory with both. In the presence of gross intestinal ulcerations more extensive therapy seems to be indicated. Similar unsatisfactory experience in acute amebic dysentery has been observed by Armstrong, Wilmot and Elsdon-Dew (1950) using Aureomycin for 15 days. One month after treatment they found that seven of 20 patients were failures. That this may be related to secondary bacterial invaders which along with the amebae are not eliminated by therapy terminating before the absolute healing of ulcers is suggested by the work of Hargreaves (1945). He found that cases of resistant amebiasis responded if first given a course of penicillin and sulfasuxidine. It appears that regardless of the antiamebic agent used, a single course of treatment frequently fails to eradicate amebae although it may produce symptomatic cure. Prolongation of a single course of therapy for a period beyond the stage of complete healing of the ulcerations might be effective. In such circumstances one might anticipate a cure rate similar to that of asymptomatic cyst passers.

Satisfactory evaluation of response data from patients with nondysenteric and relatively asymptomatic amebiasis is very difficult. Aside from the side

effects of fumagillin at the dosages used in this study, there was no observed difference between the two series of patients.

With adequate dosage, both groups became negative for *Endamoeba histolytica* within three days and there were no recurrences during hospitalization. Of seven fumagillin-treated patients relapsing during hospitalization (Table 1), five received what is now considered inadequate dosage and two were patients with dysentery. Similarly, eliminating the patients with dysentery, there were no recurrences in the hospital among patients receiving oxytetracycline.

When this study was initiated fumagillin had not been administered in quantities larger than 5 mg. daily and even then only for toxicologic purposes. Hence, in the present investigation, dosage was gradually increased until almost all patients showed side reactions that precluded its use in such quantities for routine treatment. From the patient's point of view, anorexia was the most undesireable feature of fumagillin therapy. This became particularly apparent in doses above 50 mg. daily. Below 50 mg. relapses occurred in the hospital. From this it appears that 50 mg. daily is the optimal dosage.

Even at 50 mg. daily there are side effects which militate against its use for the ordinary patient. It seems probable that, as suggested by Anderson (1952), it will have its greatest value in the treatment of chronic, drug refractive, intestinal amebiasis. Its routine clinical use in amebic dysentery cannot be justified in spite of its effectiveness in some patients. Of course, a single drug which controls dysentery and produces a satisfactory rate of parasitologic cure is not yet available. A simultaneous two-fold attack against the ameba, directly with fumagillin in the dosage of 50 mg. daily, and indirectly through the necessary bacterial associates using oxytetracycline would seem more promising. This is currently under investigation, but with the awareness that for extra-intestinal complications, other drugs as chloroquine or emetine may have to be added at times.

SUMMARY

Of 115 patients treated for amebiasis, 67 were given fumagillin and 48, oxytetracycline. Patients with dysentery are considered separately because of differences in severity of illness, the development of extra-intestinal lesions, slower response and occurrence of relapses while still hospitalized.

Among 13 patients with dysentery receiving fumagillin, two developed hepatic abscess while under treatment. One of the patients with abscess died although receiving concomitant chloroquine. The other recovered after change of therapy to oxytetracycline and chloroquine. Two had recurrence of amebae while still hospitalized.

Sixteen patients with dysentery received oxytetracycline. Two developed hepatic abscess on treatment and one presumably had an unrecognized abscess on admission. The latter died on the sixth day of oxytetracycline therapy. Each of the remaining two received concomitant chloroquine. One recovered rapidly; the other died.

Of 86 patients with nondysenteric amebiasis, 54 were treated with fumagillin and 32 with oxytetracycline. On adequate dosage, there were no recurrences in

either group during a minimal post-treatment period of 4 weeks in the hospital. Excluding the side effects of fumagillin, there were no significant differences in parasitologic or clinical response.

The environmental conditions to which these patients were exposed after release from the hospital are such as to render impossible an accurate evaluation of cure rates on the basis of prolonged follow-up.

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AN ELECTRON MICROSCOPE STUDY OF *TOXOPLASMA*

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Although *Toxoplasma* has received considerable attention from a great many authors, little morphological detail has been added to the original reports of Nicolle and Manceaux (1908, 1909) describing this organism. This is largely a reflection of its small size, its refractivity when unstained, and its generally poor staining characteristics. Small advantage is gained by variation of fixation and staining for light microscopy, or by use of the phase-contrast microscope.

Electron microscope study of total specimens of *Toxoplasma* has been generally unrewarding. The use of thin sections, however, has revealed a wealth of structural detail and serves as the basis for this report.

Historical survey. Nicolle and Manceaux (1908, 1909) described this parasite as crescentic, ovoid, or rounded; 4–7 microns long and 2–4 microns wide; occurring intracellularly or extracellularly, singly, in pairs, or in clusters; heteropolar, both ends being somewhat pointed, but one more than the other. The nucleus is round, variable in position but usually nearer the blunt end, not vesicular, and not influencing the shape of the cell. Occasional specimens show extranuclear granules, variable in size and shape, which are not kinetoplasts. The organism is nonmotile and no flagellum nor vestige thereof is present. Division is by longitudinal bipartition. This basic description has been repeated in substance by many other authors.

Chatton and Blanc (1917) mentioned a "paranuclear" region between the nucleus and the acute end consisting of an area of intensely staining granules or a large vacuole. They also pointed out that the nucleus, stained with Giemsa's stain, is diffusely granular, but when fixed with Bouin-Duboscq's solution and stained with iron hematoxylin, it consisted of a dense central body within a clear nuclear space. The pointed end of the cell was said to be emphasized by staining and beaklike in the more slender specimens.

A recent extensive cytological study of *Toxoplasma* (Cross, 1947) includes a description of a "cytostyle", a longitudinal cytoplasmic inclusion just below the cell membrane, and a "phlange", a relatively clear, finlike, longitudinal protrusion of the cell surface, sometimes containing fat droplets. Cytoplasmic granules were variable in presence and position, some siderophilic, others eosinophilic, but none giving a positive test for volutin. A relatively constant granule was observed near the blunt end.

The reports of flagellated forms (Splendore, 1913; Cross, 1947) "probably have another explanation" (Weinman, 1952).

The use of phase contrast microscopy has been mentioned by a few authors but no major advances have resulted from its application. Weinman (1952) reported that this device enabled him to see a structure probably corresponding to Cross's cytostyle in unfixed cells. Lelong and Desmonts (1951) found normal

cells too refractive and homogeneous for this technique but the organisms influenced by antibodies or other adverse treatment showed more internal differentiation, especially in granularity.

Bringmann and Holz (1953) attempted the use of the electron microscope to study whole specimens of *Toxoplasma*. They found that the normal cells, either fixed or unfixed, were too thick and opaque to determine any internal detail. Their procedure of suspending the unfixed organisms in hot distilled water to gain transparency introduced many distortions in both internal and external characteristics without compensating advantages. Our early efforts using a similar procedure confirmed these results.

MATERIALS AND METHODS

The *Toxoplasma* used in this investigation was derived from the RH strain (Sabin, 1941) obtained from the USPHS Rocky Mountain Laboratory. When maintained in white mice, it appears to be identical with the strain as originally described.

Both peritoneal exudate and minced liver tissue were tried as vehicles for the *Toxoplasma* to be sectioned. The peritoneal fluid appears to have definite advantages in both fixation and concentration, and has been used exclusively for the material reported here. Exudates were pooled from several mice four days after intraperitoneal inoculation. Two variations in preparation for fixation were found useful. At first, sediment from the centrifuged fluid was imbedded in melted 10% gelatin in saline, which was then cooled, cut into cubic millimeter blocks, and immersed in the fixative (Kautz and DeMarsh, 1954). Portions of the blocks proved to be excellent but fixation was too often inconsistent, and concentration of the *Toxoplasma* was sometimes inadequate. More recently we have injected the peritoneal fluid directly into the fixative, thus decreasing the elapsed time before fixation, increasing the uniformity of the material, and producing a flocculent sediment easily concentrated.

The fixative in all cases was an aqueous solution of osmium tetroxide buffered at pH 7.4 with veronal-acetate (Palade, 1952). Variations of time and concentration were compared, and an apparent optimum was found using 0.5% OsO_4 for two hours at 4°C. The addition of 0.32% NaCl to the buffered fixative decreased vacuolization of the cells. After washing and dehydration with graded alcohols, the sediment was impregnated in a mixture of 4 parts butyl methacrylate monomer and 1 part isobutyl methacrylate monomer. Polymerization in No. 2 gelatin capsules was carried out at 48°C. with the aid of a catalyst (Newman *et al.*, 1949). Thin sectioning was accomplished with a modified Spencer rotary microtome (Pease and Baker, 1948) using thermal expansion (Newman *et al.*, 1949), and glass knives (Latta and Hartman, 1950). Sections were collected in a 50% acetone-water reservoir behind the knife edge and picked up directly on formvar-coated 200-mesh stainless steel grids. The sections were screened by microscopic examination under reflected light; those showing interference colors indicating a thickness of approximately 0.05 micron or less were chosen for study. The sections were studied in a RCA model EMU-2B electron microscope equipped with

a self-biased gun and limiting aperture in the objective pole piece. Selected specimens were photographed routinely at an initial magnification of 3800×, and further enlargements made from these negatives.

OBSERVATIONS

The sections viewed in the electron microscope contain random associations of exudate cells, free toxoplasmas, and noncellular debris. As many as several hundred parasites may be seen on a single grid. They may be recognized by their shape, size, relationship to exudate cells, and inclusions. Round or oval contours, representing cross or oblique sections, are most common. Longitudinal sections are much less crescentic than the living organisms, partly from the orientation of the cell, but also suggesting contraction at the time of fixation.

The host cells in the exudate are largely mononuclear although some polymorphonuclear cells occur. A large proportion of all these white cells contain *Toxoplasma* in numbers varying from one to a dozen in a section. The parasites are held within vacuoles which have discrete boundaries. There is a definite space between the parasite and the vacuole wall, in many cases containing a fine filamentous or granular precipitate. Often concentrations of mitochondria may be seen at the edges of the vacuoles, while other portions of the cytoplasm will be devoid of these structures (Figures 1, 3). Occasionally the nucleus is similarly situated adjacent to a vacuole (Figure 1). Most of the parasitized cells show surprisingly little deterioration; the extensive vacuolization seen in infected tissue culture cells is usually absent. In general, fixation of the white cells differs from that of the parasites: the white cells are "overfixed" when the toxoplasmas have an optimum texture.

The outside contour of the parasite is well marked by a cell membrane without a cell wall. The surface is generally smooth but in certain oblique cuts a longitudinal fibrillar component of the surface can be seen (Figure 19). Whether these structures are imbedded in the surface membrane or project as external ridges is not clear. In some preparations, a second layer appears below the surface membrane. There is no indication of flagella, cilia, or other obvious projections on any cell. Cross-sections fail to reveal the "phlange" described by Cross (1947). An interesting variant in the cell contour is demonstrated by certain individuals which have a conspicuous indentation at the blunt end (Figure 17).

The most striking finding of this study has been the demonstration of elements involved in the polar organization in both elongate and rounded cells. The acute end shows a very distinct organelle (Figures 2, 4, 7–13, 15), which is a hollow, truncate cone 0.15–.25 μ in diameter and 0.2–.3 μ long. The base of the cone, which is toward the center of the cell, is open to the adjacent cytoplasm, but it is not clear whether the distal end, closely associated with the cell membrane, is perforate. In occasional extended cells it tends to protrude slightly (Figures 4, 7, 12) but more often it is somewhat retracted into the cell substance (Figures 13, 15). This structure will be referred to as the "conoid".

Associated at one end with the conoid and following a longitudinal course through the cell are several long, roughly cylindrical bodies of a dense, homo-

geneous material (Figures 2, 4, 11, 14). Their cross-sections are uniformly circular. No surface membrane appears to be present. Maximum diameters are 0.08–.12 μ. Total lengths appear to vary greatly; some extend nearly to the blunt end of the cell (Figures 2, 4) and others cannot be followed as far as the nucleus. As they approach the conoid, they become very slender and tortuous, and can be seen to converge, entering into the base of the cone (Figures 2, 3, 11). Usually they are more or less parallel (Figure 2), but sometimes a few diverge sharply (Figure 4). The appearance of several disks in a compact group is interpreted as a cross-section of a bundle of these structures (Figures 5, 6). This grouping can sometimes be found in the blunt end of the cell, indicating that it may be maintained throughout the length of the cell. From longitudinal cuts, as many as 8 to 10 separate members can be seen (Figure 2). In cross-sections, from 14 to 18 individuals occur. The total number may be variable with a mean of about 16. We suggest the term "toxoneme" for these filamentous structures which appear to be peculiar to *Toxoplasma*.

A variety of other cytoplasmic inclusions are commonly seen. Isolated dense round bodies (Figure 6), not in a definite pattern like the toxonemes, may represent vacuoles of some osmophilic substance, such as a lipid. Configurations of fine granules in a cluster against the nucleus (Figures 6, 14, 17) have been found in many preparations. These occur in a position which might agree with that of a centriole or the Golgi apparatus, but do not resemble illustrations of these two components presented by other workers (Dalton and Felix, 1953; Beams and Tahmisian, 1953). Occasionally a more compact group of granules is seen in a similar juxtanuclear position (Figure 15). Bodies suggesting mitochondria occur commonly in compact formation (Figures 5, 6) or dilated after use of a hypotonic fixative, with projections similar to cristae (Palade, 1953) arising from their walls (Figure 12). In a few pictures, small fragments of canalicular inclusions are seen (Figure 18). These agree in dimension and conformation with the endoplasmic reticulum as depicted by Palade (1952), but further study is required to confirm this impression.

The nucleus appears distinct in a large proportion of the *Toxoplasma* sections. It is usually round or slightly oval in outline, but may be elongated transversely or longitudinally, or prominently lobed. Round contours often have one flattened side (Figures 6, 17). An unusual but not unique finding is the horseshoe shape seen in one member of Figure 13 and in Figure 16. The position of the nucleus agrees with previous observations: it is found in the middle third of the cell, usually somewhat toward the blunt end. Its diameter is about 1 to 1½ μ when circular, and up to 2 μ in the greatest dimension when elongated.

There appears to be a definite nuclear membrane with a thickness of approximately 10 mμ. Where the preparation has caused separation of the nucleus and surrounding cytoplasm, there is a discrete surface membrane of the cytoplasm as well. Most of the nuclear membrane has applied to its inner surface a dense, irregular layer. Masses of a similar density occur within the nucleus but the position, size, and dimensions are quite variable.

No stages have yet been seen which would agree closely with patterns of

mitosis such as were depicted by Chatton and Blanc (1917) and Cross (1947). The lobed, elongated and horseshoe shaped nuclei (Figures 6, 13, 16, 17) may be stages of division whose interpretation is obscure at present. In the relatively uncommon associations of paired cells showing cytoplasmic division (Figure 11) the nuclear division is already complete.

DISCUSSION

Electron microscopy of thin sections for studying the morphology of this organism has proved to be especially useful for a number of reasons. It can be obtained in large numbers and concentrated easily. Lack of a cell wall allows good permeability and a soft texture for sectioning. Its size is such that even the larger inclusions, such as the nucleus, are difficult to see well with light microscopy, but it still exhibits a high degree of organization when studied with the electron microscope.

The host cells lend themselves well to study by this system. Nuclei and cytoplasmic granules can be used to identify the cell types of the exudate. Eosinophile granules are more common than would have been expected from stained smears of the material. The terminal colonies ("pseudocysts") did not appear in many preparations; probably they were shattered by the manipulation required for withdrawal of fluid and fixation. The host cells that remained intact were those whose substance showed little of the deterioration that can be seen in infected living cells of a tissue culture. The concentration of mitochondria at the edge of the vacuoles containing the parasite clusters is notable. An explanation of this phenomenon is lacking but it appears that the migration is that of the mitochondria rather than of the parasite, since other parts of the host cells are devoid of these structures.

As might have been predicted, many of the details demonstrated in the morphology of this organism are quite similar to those of a variety of other cells. The nucleus conforms to the pattern of a discrete body bounded by a distinct membrane, contrary to the early opinion of Sabin (1942). The clumps of dense material within the nucleus show some indication of a consistent large mass (Figures 1, 13, 14, 16), but not similar to the central concentration found after Bouin-iron hematoxylin nor the course granulation and bipolarity after methyl alcohol-Romanowski techniques. Cytoplasmic inclusions are variable and include both round and filamentous elements. Small mitochondria are seen with enough regularity to assume that they are a normal constituent of the cell. Identification of the granules (siderophilic, iodophilic, etc.) seen by light microscopy with those seen under these circumstances will require precise mechanisms of correlating the two procedures. Absence of a cell wall or pellicle reinforces the impression of flexibility gained from observation of the viable cells.

Although the conoid has not been described before, the presence of such a structure at the pointed end was anticipated by Chatton and Blanc (1917) and Cross (1947). The illustrations of Bringmann and Holz (1953) show the increased density in this area, but these authors made no comment concerning it as an anatomical feature. The bundle of toxonemes probably agrees with the para-

nuclear area of Chatton and Blanc. Whether it is to be identified with the cytostyle of Cross (1947) is not clear.

The peculiar pattern of the conoid and the toxonemes invites speculation as to their function. There appears to be no close anatomical parallel described at present in other protists, although if the dimensions of the elements were in the same range as those of *Toxoplasma*, their detection would depend on electron microscopy. Manwell (1953) indicated that the acute end of the cell is anterior during its movement. The presence of the conoid at the anterior end would be significant. Among the several possibilities that can be suggested must be included the designation of the conoid as a mouth structure, a penetration device, or a vestige of protozoal structure such as a cytopharynx. The association of the toxonemes then might imply secretory mechanisms for nutrition or penetration, or perhaps modifications of structures related to locomotion such as a kinetoplast. The absence of any external or internal structure which might be identified as a flagellum makes the latter alternative more remote. The number and arrangement of the toxonemes does not agree with that of the polar filaments of Myxosporidea nor with the trichocysts of ciliates.

Although it had been hoped that this study might contribute to the solution of the perplexing problem of classification of *Toxoplasma*, no easy resolution is in view. Since the most distinctive parts of the organization of this parasite find no counterparts in the description of organisms suggested as possibly related, it would be of considerable interest to investigate these with a similar technique. Such a list would include *Encephalitozoon*, Sarcosporidia, tissue forms of *Histoplasma*, the cryptozoites of *Plasmodium* and other Hemosporidia, *Leishmania*, and perhaps other less parasitic forms such as *Labyrinthula*. Investigations relative to some of the less obvious phases of this study are still in progress.

SUMMARY

Electronmicrographs of thin sections of *Toxoplasma* reveal morphological details hitherto unsuspected from study by traditional techniques. Examples of nuclear and cytoplasmic patterns are described and depicted. The structure of the end-organelle and some peculiar longitudinal inclusions associated with it does not find a parallel among descriptions of other protists.

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PLATE I

Reference line 1 μ

FIG. 1. Section through a large mononuclear cell showing numerous *Toxoplasma* in vacuoles. Mitochondria line the vacuole walls. Several typical nuclear patterns and cytoplasmic inclusions are visible.

1μ
1

PLATE II

Uniform magnifications. Reference line 1 μ (Fig. 4)

FIG. 2. Longitudinal cut through two intracellular *Toxoplasma*, one of which demonstrates the conoid and a bundle of eight or more toxonemes.

FIG. 3. Detail from Fig. 1. An oblique section through the acute end of two *Toxoplasma* cells shows the slender ends of toxoneme elements passing through the base of the conoid. Note the mitochondria of the host cell.

FIG. 4. Section showing a conoid, and toxonemes with diverging pathways.

FIG. 5. An intracellular organism depicting the cross-section of a bundle of 17 ± toxonemes in a typical pattern, and a small mitochondrion.

FIG. 6. Extracellular organisms showing nuclei varying from the round pattern. Cytoplasmic structures include mitochondria, cross-section of a bundle of 15 ± toxonemes, and juxtanuclear granules.

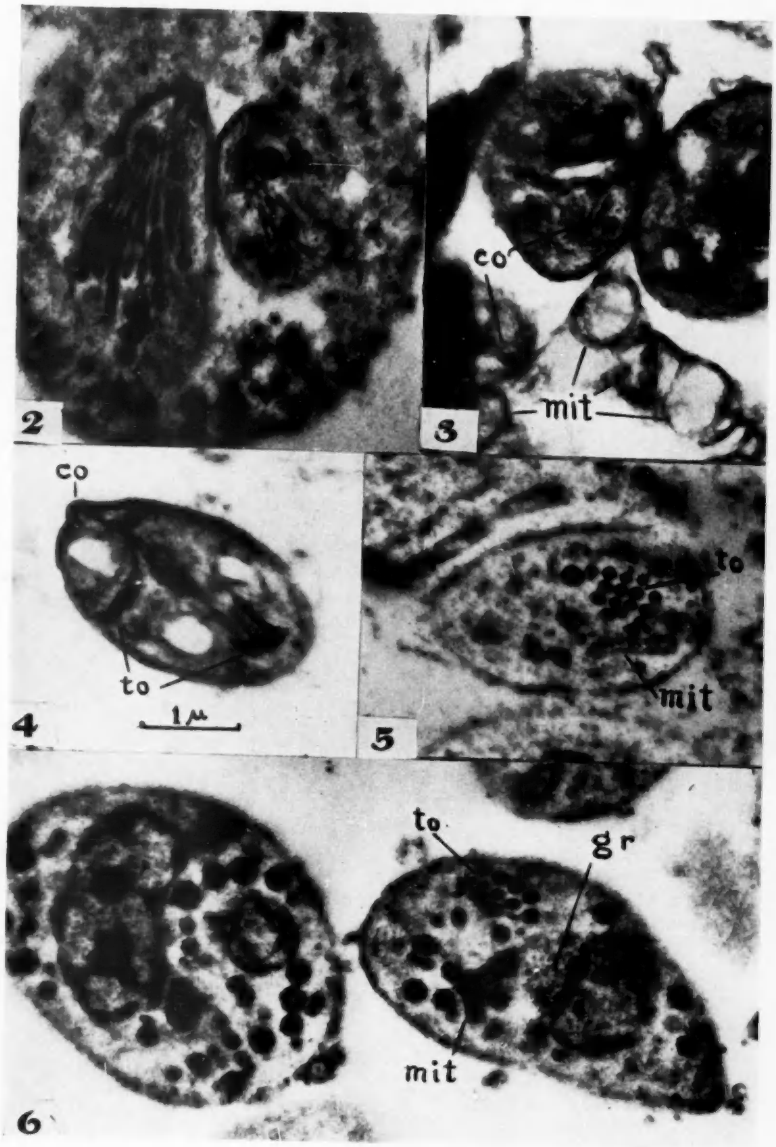

2
co
mit
3
co
to
1μ
4
to
mit
5
to
gr
mit
6

PLATE III

Uniform magnifications. Reference line 1 μ (Fig. 11)

FIG. 7. Acute end of a cell with a longitudinal section of a conoid.

FIG. 8. Conoid in oblique section.

FIG. 9. Conoid in nearly transverse section with dense central concentration of toxonemes.

FIG. 10. Cross-section of acute end, with conoid represented as a circle. Radial lines are surface markings.

FIG. 11. Intracellular *Toxoplasma* in late stage of division. Nuclei of the daughter cells are distinct, but the cytoplasmic division is not yet complete. The conoid of one of the cells is 90° from the point of attachment.

FIG. 12. Extracellular organism with protruded conoid and dilated mitochondria.

FIG. 13. Cluster of extracellular *Toxoplasma*. Nuclei are rounded in two, and horseshoe shaped in one.

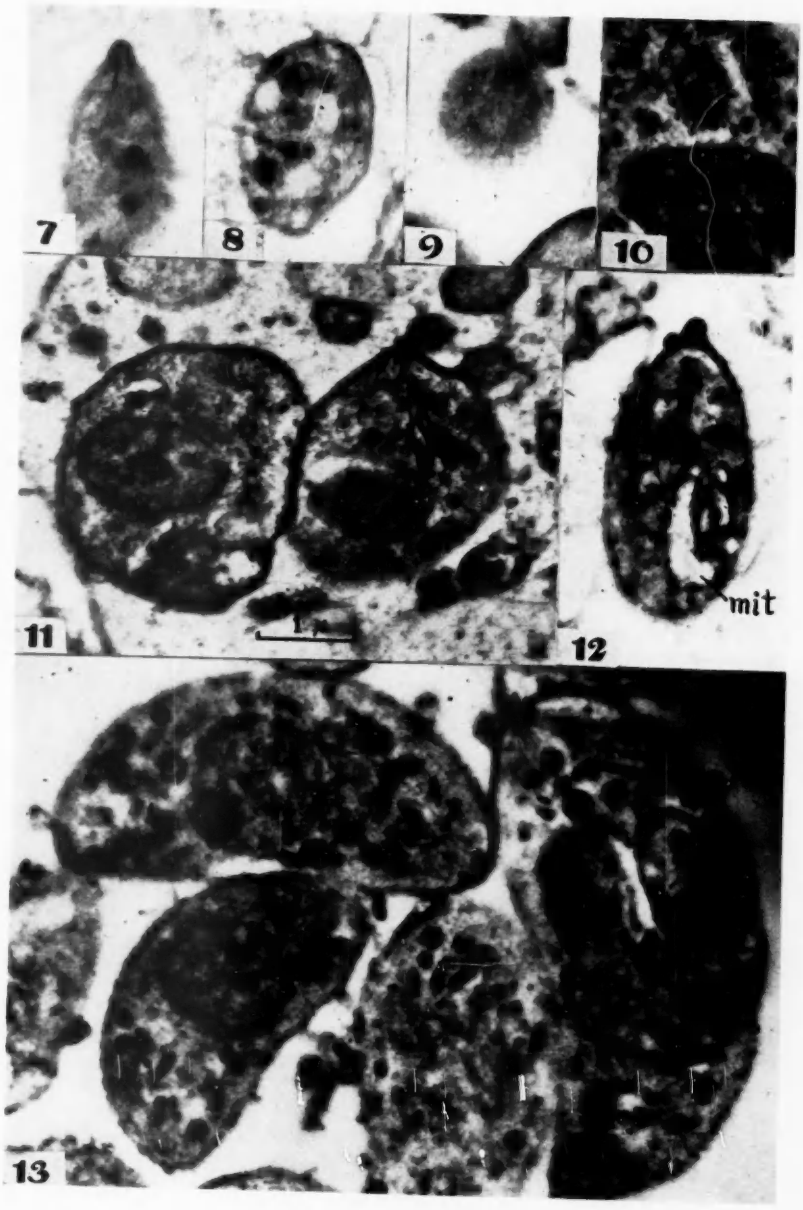

7
8
9
10
11
12
mit
13

PLATE IV

Reference lines 1 μ

FIG. 14. Paired extracellular *Toxoplasma* showing varying nuclear patterns, toxonemes, and juxtanuclear granules.

FIG. 15. A small compact group of granules appears adjacent to the nucleus.

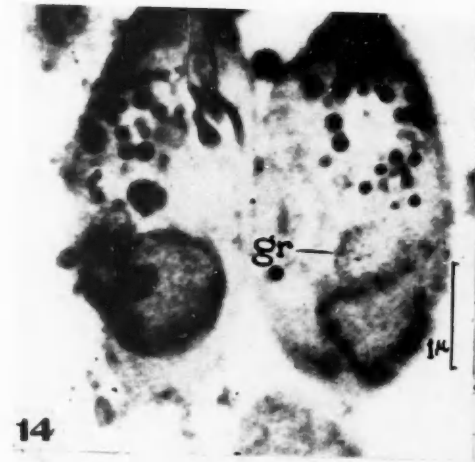
FIG. 16. An extracellular cell with a horseshoe shaped nucleus.

FIG. 17. An organism with a deep indentation at the blunt end of the cell.

FIG. 18. Intracellular parasite showing canalicular inclusions suggesting endoplasmic reticulum.

FIG. 19. Oblique cut showing surface marking.

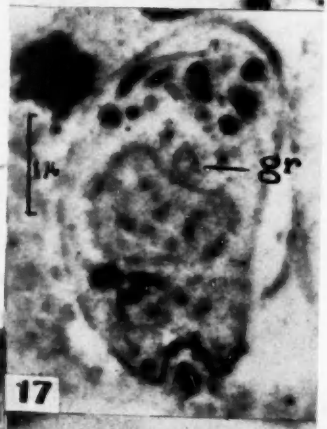
Abbreviations: co = conoid
to = toxoneme
mit = mitochondria
gr = juxtanuclear granules
gr′ = compact juxtanuclear body
er = endoplasmic reticulum-like structures
s = surface markings



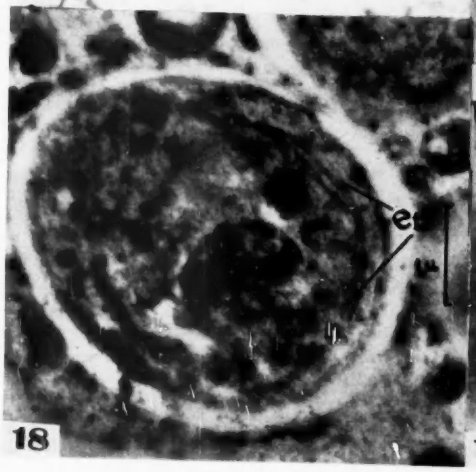
14

15

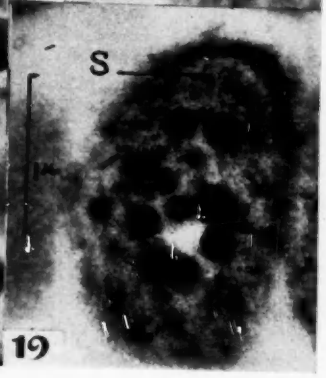
16



17



18



19

SPONTANEOUS TOXOPLASMOSIS IN THE WHITEFACE MONKEY, *CEBUS CAPUCINUS*, IN PANAMA

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Spontaneous toxoplasmosis in primates other than man was first reported by Thezé (1916) in a monkey, *Stentor seniculus*. Later Levaditi and Schoen (1933) described a natural infection in *Cynocephalus babuin* and Kopciowska and Nicolau (1938) in a chimpanzee. This work has been ably reviewed by Cowen and Wolf (1945). In the present communication spontaneous toxoplasmosis in a Panama whiteface monkey, *Cebus capucinus*, is described. The strain of organism was isolated in white Swiss mice and has been maintained to date by continuous passage in this rodent.

An infant whiteface monkey was brought into the laboratory in a moribund condition in August of 1953 by a local dealer who requested us to examine it for possible yellow fever. This dealer purchased large numbers of monkeys for export and maintained them for variable periods of time in screened enclosures on the outskirts of the city. He complained of heavy losses among the animals recently. The *Cebus* brought in had received one injection of penicillin the previous day.

The monkey, an emaciated infant, was sacrificed. No gross pathological changes were observed except for a small pneumonic focus in the right upper lobe of the lung. Parasites were not observed in an ordinary thick film of the blood nor in fecal suspensions. Blood cultures yielded growth of *Pseudomonas aeruginosa*. Six mice were inoculated intracerebrally with the serum of this monkey and six, also intracerebrally, with a suspension of pooled sections of the cerebral cortex and cerebellum.

In one histological section of the cerebral cortex stained with hematoxylin and eosin a small toxoplasmic pseudocyst was found. The mice were kept under daily observation. Three weeks after inoculation the group receiving brain tissue all showed irritability, ruffling of the fur and light tremors. Four of these six animals were sacrificed during the following week. Enlargement of the spleen was observed in all four, congestion of the lung in two. Small numbers of *Toxoplasma* were identified in lung and brain tissue. Sections of brain, spleen and liver were emulsified for further passage in mice. One of the 6 mice inoculated intracerebrally with serum was observed 10 weeks later to carry the head tilted to one side and to move in circles. It was sacrificed and showed small numbers of *Toxoplasma* in smears of the brain.

This strain has been maintained for 24 continuous passages to date in mice. The first passages were made by the combined intracerebral and intraperitoneal injection of tissue suspensions (brain, liver, spleen and lung). A chronic type of infection with a minimum incubation period of 2 weeks was produced in these earlier passages. The spleen was greatly enlarged and the lungs showed moderate to severe involvement. Toxoplasmas were found with greatest frequency in lung

and brain tissue. In brain tissue they usually appeared in the pseudocyst form. In later passages inoculations have been performed only by the intraperitoneal route, using peritoneal fluid or washings as the inoculum. The majority of mice now sicken within 4 to 5 days and die within 7 days. An occasional mouse has survived for a month or longer presenting the chronic form of the infection. The peritoneal fluid ordinarily contains great numbers of toxoplasmas in the early acute phase.

The organisms isolated from this monkey show the same morphological characteristics as other strains of *Toxoplasma* (Sabin, 1943; Jacobs, 1953). In fresh wet preparations no active motility has been observed. They are small crescent-shaped organisms, usually pointed at one extremity and more rounded at the other, about 4 to 7 micra in length by 2 to 4 micra in width. Actively dividing forms are much wider than resting forms and are frequently oval in shape. In Giemsa-stained smears the cytoplasm is blue and the nucleus, usually located nearer to one extremity, is red or purple. We found no evidence to indicate reproduction by any other process than binary fission. The protozoons were found free or intracellularly placed, singly, in small intracellular clusters or in pseudocysts of various sizes. In the pseudocysts there was no sharp demarcation between the cytoplasm of the various individuals. In histological sections the parasites are smaller, less crescentic and less readily identifiable than in Giemsa-stained smears. No growth was obtained in ordinary bacterial culture media or in Sabouraud's, Cleveland and Collier, or NNN media.

In order further to establish the identity of the organisms isolated in mice, their pathogenicity in a variety of other animals was studied. As is well known, *Toxoplasma* is unique among parasites in its very wide range of experimental hosts. Four hamsters, two young rabbits and two guinea pigs were inoculated, all by the intracerebral route. The hamsters developed an acute meningoencephalitis with death in 4 to 5 days after inoculation. The two rabbits died in 12 and 21 days respectively and one of the 2 guinea pigs in 15 days. *Toxoplasma* were observed in the meninges and cerebral cortex in each instance. The other guinea pig survived during a ten-week period when it was accidentally destroyed. Of especial interest is the high pathogenicity of this strain for two species of small Panamanian monkeys, the marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*, in which it produces an acute uniformly fatal infection (Rodaniche, 1954). The *Toxoplasma* also grow in the chick embryo and its membranes, especially the chorioallantoic.

The immunological relationship between this and other strains of *Toxoplasma* has not yet been determined. This phase of the work must await receipt of suitable control sera for serological tests and a control strain of the parasite for cross-immunity tests.

DISCUSSION

Spontaneous toxoplasmosis has been described previously on the Isthmus of Panama in man by Kean and Grocott (1947, 1948) and Mantz and co-workers (1949), in the pigeon by Johnson (1944), in the guinea pig by Rodaniche and Pinzon (1949) and in the dog by Grocott (1950). It is now described for the

first time in the whiteface monkey. Unfortunately, we have no information as to the source of the infection—whether it was contracted in the jungle or in captivity. The strain of *Toxoplasma* here described corresponds closely in morphology and animal pathogenicity with those studied by other authors. It shows no progressive motility in fresh wet preparations and fails to grow in artificial culture media. Although it gives the same types of serological reactions as other strains of *Toxoplasma*, the establishment of its immunological identity with these strains must depend on the acquisition of suitable control material. The exact relationship of the *Toxoplasma* to cause of death in this infant monkey was not ascertained. The animal was very poorly nourished. Sections of the previously mentioned focus of interstitial pneumonia in the lung and of apparently normal liver tissue stained with hematoxylin and eosin failed to reveal structures that could be interpreted with certainty as *Toxoplasma*. One pseudocyst was encountered in a section of cerebral cortex. Other tissues and organs were not preserved. There was invasion of the blood stream as indicated by the positive result obtained in a mouse injected with the blood of this monkey.

CONCLUSIONS

Spontaneous toxoplasmosis in an infant whiteface monkey, *Cebus capucinus*, in Panama is described. This is the first report of natural infection in this species of primate.

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SUSCEPTIBILITY OF THE MARMOSET, *MARIKINA GEOFFROYI*, AND THE NIGHT MONKEY, *AOTUS ZONALIS*, TO EXPERIMENTAL INFECTION WITH *TOXOPLASMA*

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Relatively few primates have been tested for susceptibility to *Toxoplasma gondii* and those tested have usually shown a high degree of resistance. Negative results were obtained in the following species, *Cercopithecus patas* and *Papio sphynx* by Levaditi and co-workers in 1929, *Cercopithecus sabaeus* and *Papio doguera* by Cowen and Wolf in 1945, *Cynocephalus babuin* by Levaditi and Schoen in 1933 and *Macacus cynomolgus* and *Macacus sinicus* by Nicolle and co-workers in 1909 and 1913. No symptoms were produced and no toxoplasmas found in the tissues by histological examination in the few instances where this was attempted. Only very small numbers of animals, usually one of each species, were used in these experiments. *Macaca mulatta* has been tested more extensively than the other species (Levaditi and co-workers, 1929; Sabin and Olitzky, 1937; Sabin and Ruchman, 1942; Cowen and Wolf, 1945). Levaditi and co-workers obtained totally negative results in a rhesus monkey inoculated intracerebrally. Sabin and co-workers produced self-limited febrile reactions associated with parasitemia and local lesions in *M. mulatta* inoculated by various routes. Cowen and Wolf reported the first fatal infection in a 7-month old rhesus inoculated intracerebrally. No evidence of infection was obtained in 8 others except for a changing serology in 4.

It may be of interest, therefore, to report the high susceptibility to toxoplasmosis of 2 species of primates native to Panama, the marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*. In both species *T. gondii* produces a rapidly progressive fatal infection with parasites abundantly present in most of the tissues and organs at the time of death.

MATERIALS AND METHODS

The strain of *Toxoplasma* used was one recently isolated in mice from a spontaneous infection in an infant *Cebus* monkey. It had received two passages in mice prior to being tested in the marmoset. This strain has been described in another report (Rodaniche, 1954). Morphologically, in animal pathogenicity, and in physiological properties it corresponds with strains described by other authors. In the early mouse passages the strain showed low virulence for this rodent, producing only slowly developing chronic infections. After the first passage in the marmoset, however, the virulence was greatly increased for mice and acute, rapidly fatal infections were produced.

A total of 31 marmosets and 15 night monkeys was employed in tests of susceptibility. The marmosets were juvenile or adult animals weighing from 340 to 555 grams. All but 5 showed spontaneous infection with microfilariae or tryp-

anosomes of little or no pathogenicity. Such infections are very common in marmosets in Panama (Clark, 1931). No difference was observed in the course of the toxoplasmic infection in those animals free of blood parasites and in those harboring them. The night monkeys also were juvenile or adult animals, weighing from 535 to 925 grams. Repeated blood smears revealed microfilariae in one of the animals. All the others were free of blood parasites.

Inocula consisted of peritoneal fluid or tissue emulsions of infected mice or monkeys. The infection could be transferred readily from mice to monkeys and vice versa. Various routes of inoculation were tried. Both marmosets and night monkeys were tested by the intraperitoneal, intracerebral, intradermal, intranasal, ocular and oral routes. Marmosets alone were tested by the subcutaneous and intrapleural routes. Rectal temperatures and thick blood films were taken daily. Animals either were allowed to die spontaneously or were sacrificed when moribund. A postmortem examination was made of each animal and Giemsa-stained smears of the body exudates and viscera examined. In a few instances, histological sections stained with hematoxylin and eosin also were prepared.

The course of the infection in both *Marikina* and *Aotus* on the whole was similar. Temperature changes in the marmosets were variable, some animals showing only a terminal drop in temperature and others one to three days of fever, usually shortly prior to death. In the night monkey, on the other hand, fever was a constant feature, temperatures of 104°F. or higher being registered for 1 to 4 days prior to death. Both marmosets and night monkeys remained otherwise asymptomatic until about one day prior to death when they became inappetent, sluggish and tended to maintain a crouching position. Marmosets sometimes developed a cough shortly prior to death. Thick blood films in both species became positive for *Toxoplasma* within 12 to 36 hours of death. The toxoplasmas were never very numerous in the blood, usually about one to every 2 to 10 fields. Their presence in thick films could be interpreted as indication of a fatal outcome within 36 hours.

Gross pathological changes in the internal organs depended partly on the route of inoculation and partly on the duration of the infection. The lungs always showed some degree of involvement, except after intracerebral inoculation, varying from congestion to extensive bronchopneumonia. Massive edema of the lungs was a common finding. Small amounts of pleural fluid were often present. The spleen was usually enlarged, the liver congested. In marmosets intraperitoneal inoculation resulted in the formation of relatively small quantities of a yellowish rapidly coagulating peritoneal fluid. In the night monkey 5 to 10 ml. of fluid was present at the time of death and there were fibrinous adhesions between the omentum and the viscera. Local changes observed after intradermal and ocular inoculation will be described in connection with results obtained by inoculation by these routes. In animals dying of the infection, toxoplasmas were always found in very large numbers in impression smears of the spleen, liver and lungs, in smaller numbers in blood, kidneys and mesenteric lymph nodes. In the other viscera and exudates they were sometimes encountered and sometimes not, being observed at one time or another in smears of the bronchi, trachea,

esophagus, stomach, pancreas, bile, urinary bladder, testes, ovary, uterus, brain, meninges, peritoneal fluid, urine, ocular and nasal exudates, sputum and on one occasion in the milk of a lactating night monkey. There was not much correlation between the route of inoculation and the postmortem distribution of the parasites, except that they were observed in the brain only after intracerebral inoculation, in the peritoneal fluid only after intraperitoneal inoculation or subcutaneous inoculation in the inguinal area, in the skin only after intradermal inoculation and in the conjunctiva and ocular exudates only after ocular administration. Toxoplasmas were never encountered in smears of the feces or smears or sections of the intestinal mucosa. The protozoons were usually observed as plump, actively dividing forms, free or in an intracellular position, single or in small or medium-sized clusters. Pseudocysts were encountered only in the conjunctivae of 2 marmosets inoculated by the ocular route.

A summary of results of the inoculation of *Marikina geoffroyi* by various routes is presented in Table 1 and of *Aotus zonalis* in Table 2. With the exception of one *Aotus* accidentally destroyed in 3 days, all marmosets and night monkeys receiving parenteral injections of *Toxoplasma* suffered a rapidly progressive, uniformly fatal infection, with these parasites abundantly present in smears of the various tissues and organs. Death occurred in 3 to 11 days.

Intranasal inoculation, performed by dropping 2 to 5 drops of the inoculum in each nostril, resulted in a fatal toxoplasmic infection in 2 of 3 marmosets in 10 and 11 days respectively. The third marmoset died of intercurrent infection in 6 days. Toxoplasmas were not encountered in tissue smears of this animal. One of 2 night monkeys similarly inoculated succumbed to the infection in 13 days, the other was accidentally killed in one day.

Oral administration, performed by allowing one to two ml. of the inoculum to

TABLE 1

Susceptibility of Marikina geoffroyi *to inoculation with* Toxoplasma *by various routes*

Route of Inoculation	No. of Monkeys Used	No. Dying	Days of Death	No. Showing *Toxoplasma* in their Tissues Postmortem
Intraperitoneal	4	4	4, 5, 5, 6	4
Intrapleural	2	2	6, 10	2
Subcutaneous	4	4	3, 6, 8, 10	4
Intracerebral	2	2	5, 5	2
Intranasal	3	3	6,* 10, 11	2
By feeding	3	3	4, 4, 10	3
By stomach tube administration	4	2	1,* 13	1
Intradermal	3	3	7, 7, 11	3
By instillation into the eye	5	5	8, 9, 10, 11, 12	5
By instillation into the vagina	1	0		
Total	31	28		26

* Indicates the animal dying on that day did not show toxoplasma in the tissues, death being due to accident or intercurrent infection.

TABLE 2

Susceptibility of Aotus zonalis *to inoculation with* Toxoplasma *by various routes*

Route of Inoculation	No. of Monkeys Used	No. Dying	Days of Death	No. Showing *Toxoplasma* in their Tissues Postmortem
Intraperitoneal	3	3	5, 6, 7	3
Intracerebral	2	2	4, 5	2
Intranasal	2	2	1,* 13	1
By stomach tube administration	2	1	8	1
Intradermal	4	4	3,* 6, 8, 11	3
Conjunctival	2	1	13	1
Total	15	13		11

* Indicates the animal dying on that day did not show toxoplasma in the tissues.

run into the mouth from a pipette while the animal was maintained under ether anesthesia, resulted in death of all 3 marmosets so inoculated in 4, 4, and 10 days respectively. Toxoplasmas were readily identified in various tissues postmortem. However, administration of 2 to 4 ml. of the same inoculum by stomach tube without anesthesia produced infection in only 1 of 4 marmosets and 1 of 2 night monkeys. These two animals died in 13 and 8 days respectively and showed the parasites widely distributed throughout their tissues. One marmoset died in 24 hours of other causes. Two marmosets and one night monkey survived throughout a 2-month observation period. Complement-fixation and intradermal neutralization tests against toxoplasma were conducted with their blood at 30- and 60-day intervals with negative results. One of the marmosets and the night monkey were sacrificed at the end of the 2-month observation period. Toxoplasmas were not observed in direct smears or histological sections of their tissues nor were the parasites recovered by subinoculation of emulsions of brain, spleen, kidney and liver tissue in mice. The other marmoset was reinoculated with *Toxoplasma* by the intraperitoneal route and developed an acute infection which proved fatal in 4 days indicating that it had developed no immunity.

Intradermal injection of 0.1 to 0.2 ml. of body fluids or tissue suspensions containing variable numbers of viable *Toxoplasma* produced an acute infection in all of 3 marmosets and 3 of 4 night monkeys, with death in 6 to 11 days. The fourth night monkey was lost to intercurrent infection in 3 days. A raised red papule developed at the site of inoculation in 4 days. On the fifth day a central vesicle filled with nonpurulent fluid was formed. These vesicles remained small in the marmoset, but in the night monkey achieved a diameter of 8–12 mm. Later there was ulceration of the center and scar formation. Moderate numbers of *Toxoplasma* were found in the vesicular fluid during life and in the skin and subcutaneous tissue postmortem. The severity and rapidity of development of the skin lesions depended directly on the number of *Toxoplasma* injected, 25 or fewer organisms producing a minimal reaction in 7 days and 250–25,000 organisms a maximum reaction in the same period.

This local reaction could be neutralized by positive monkey serums in tests essentially similar to those devised by Sabin and Olitsky (1937), employing the rabbit as experimental animal. The following technique was employed. Mouse peritoneal fluid was diluted serially with saline solution to contain from 2,500 to 5,000; 25,000 to 50,000; and 250,000 to 500,000 *Toxoplasma* per ml. To 0.1 ml. of each dilution was added 0.1 ml. of immune monkey serum. The mixtures were allowed to remain one hour at room temperature. Then 0.1 ml. of each dilution was injected intradermally in the shaved abdominal skin of paired marmosets. As a control, serial dilutions of *Toxoplasma* were similarly combined with negative monkey serums and injected. Failure to develop a papule 6 mm. or more in diameter with necrotic center within 6 to 8 days of inoculation was interpreted as a positive reaction, provided typical lesions were observed in the control sites.

Intravaginal inoculation in one marmoset failed to produce infection. One ml. of a heavily infected monkey liver suspension was introduced into the vagina without trauma with a blunt pipette. The animal survived throughout a 12-week observation period when it was sacrificed. Direct smears, histological sections and subinoculations into mice of brain, spleen, liver, lung and kidney tissue failed to reveal the parasites. *Toxoplasma* were not observed in scrapings of the vaginal or uterine mucosa or impression smears of the ovaries.

Ocular inoculation proved to be of especial interest. Five marmosets and 2 night monkeys were inoculated by dropping 1 to 4 drops of the inoculum in the eyes under ether anaesthesia while gently manipulating the lids with gloved fingers. This route of inoculation produced an acute fatal infection in all 5 of the marmosets with death in 8 to 12 days, and in one of the 2 night monkeys which died in 13 days. In the marmosets, edema of the face was noted one or two days prior to death and a watery exudate filled the eyes. About one day prior to death the blood smears became positive and *Toxoplasma* were present in the ocular exudates and nasal secretions. A persistent cough was noted. Postmortem, the lungs showed the most severe changes. *Toxoplasma* were identified in large numbers in smears of the conjunctiva, liver, lung and spleen and in lesser numbers in the edema fluid present in the subcutaneous tissue of the face, in the nasal exudates, trachea, kidney and mesenteric lymph nodes. The *Toxoplasma* in smears of the conjunctivae were found free, in small intracellular groups and in two instances in large pseudocysts, the only occasions when pseudocyst formation was observed in *Marikina*. In the night monkey which succumbed to the infection there was slight opacity of the cornea, but no edema of the face or exudation of the eyes was observed. There was a bronchopneumonia involving all lobes of the lung with water-logging. *Toxoplasma* were relatively scarce in smears of the conjunctivae, but extremely abundant in smears of liver, lung and spleen. They were present also in throat and nasal swabbings. No *Toxoplasma* were found in smears of the brains of any of these monkeys.

DISCUSSION

The marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*, should prove useful experimental animals in the investigation of the numerous

unsolved problems in the field of toxoplasmosis. Both species are highly susceptible and develop an acute fatal infection after inoculation by a number of routes. No evidence has as yet been obtained of a chronic form of the disease in *Marikina* or *Aotus*, at least with the strain employed here.

Of especial interest is the susceptibility of these monkeys to infection by feeding or by instillation of the inoculum into the eyes or nose without trauma. The *Toxoplasma* are capable of direct invasion of the conjunctiva where they multiply and from which they spread producing a generalized infection and death. The marmoset is more highly susceptible to this route than the night monkey. This finding suggests the possibility that the eye may be a natural portal of entry of the disease. Local multiplication in the nasal mucosa was not noted after intranasal instillation of the inoculum. Infection by feeding is probably effected through the upper respiratory tract as only 2 of 6 monkeys showed evidence of infection after feeding by stomach tube. Irregular results by stomach tube administration were also obtained in rabbits by Wolf, Cowen and Paige (1940) and in guinea pigs by Kozar and co-workers (1952).

Our failure to infect one marmoset by a single intravaginal instillation of *Toxoplasma* obviously has a limited significance. Cowen and Wolf (1950) succeeded in infecting pregnant mice by repeated intravaginal instillations. The effect of repeated instillations in a large number of marmosets in various physiological states remains to be ascertained. *Toxoplasma* are commonly found in smears of the uterine mucosa of both night monkeys and marmosets dying of the disease although not in such large numbers as in other organs.

Preliminary experimentation according to the technique described indicates that the local reaction provoked by the intradermal injection of *Toxoplasma* may be neutralized by immune monkey serum. Further standardization is required before results with human serums can be interpreted accurately. *Marikina* and *Aotus* should prove superior to the rabbit as experimental animals for this type of reaction because resistance to infection in these animals in nature must be rare, if it occurs at all. To date we have used a total of 60 marmosets and night monkeys without encountering a single immune one.

CONCLUSIONS

The marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*, are highly susceptible to experimental infection with *Toxoplasma gondii*. They uniformly develop acute fatal infections after inoculation by a wide variety of routes.

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PATHOLOGY OF *DIENTAMOEBA FRAGILIS* INFECTIONS OF THE APPENDIX

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Although *Dientamoeba fragilis* is generally considered to be a nonpathogenic ameba, there is increasing evidence that infection with this species often is associated with clinical symptoms which do not disappear until the infection is eliminated. (Costa, 1948; Gittings and Waltz, 1927; Hakansson, 1936, 1937; Hood, 1940; Jepps and Dobell, 1918; Knoll and Howell, 1945; Mollari and Anzulovic, 1938; Rachet, Busson and Terrial, 1945; Robertson, 1923; Sapero, 1939; Thomson and Robertson, 1923; Wenrich, Stabler and Arnett, 1936; and Wenrich, 1937, 1944). Inasmuch as no fatal cases have been reported and no evidence of tissue invasion demonstrated, its status has been in doubt.

Protozoa which are pathogenic cause one or more of the following damages to the host: (a) invade and/or destroy cells or tissues; (b) produce toxic byproducts; and (c) produce a general or localized tissue reaction in the host. Death of the host may or may not result from one or more of the above activities.

During routine examinations of 581 appendices for intestinal parasites, *D. fragilis* was encountered in four patients. These four cases will be reported from the standpoint of known symptoms of the patients, the gross and microscopic pathology of the appendices and the activities of the parasites within the appendices.

PROCEDURE

Surgically removed appendices from the various Army and Air Force hospitals are received at the Sixth Army Area Medical Laboratory in ten per cent formalin. Routinely, portions from three different levels of each appendix are removed for sectioning and a part of the contents of the remaining portions is washed out in formalin solution for examination by the Parasitology Branch. In the four cases reported here, additional levels of each appendix were embedded and sectioned for a more comprehensive study.

The appendiceal contents were examined directly by formalin-ether concentration and by iron-hematoxylin staining. Prior to staining the material was fixed to the slides with PVA fixative. The sectioned material was divided into two groups of slides, one group being stained with Harris's hematoxylin for use by the Pathology Branch and the other group being stained by the tergitol iron-hematoxylin method for use by the Parasitology Branch.

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CASE REPORTS

Case 1. A 28 year-old white male had infrequent intermittent bouts of vague abdominal symptoms over a period of about seven years. At times this discomfort was associated with diarrhea. In October, 1952, the patient experienced an episode of acute, severe, knife-like pains in the right lower quadrant of the abdomen, but no nausea or vomiting. The hemogram revealed 13,900 WBC, with 78 per cent neutrophils, 22 per cent lymphocytes and no eosinophils. The appendix was removed. Early in 1953 the patient experienced similar sharp pains, now in the hypogastric region, which then progressed to the lower right quadrant. Several stool examinations, two following purgation, failed to reveal any parasites other than ova of *Trichuris trichiura.*

Case 2. A 20 year old white female experienced severe lower right quadrant adominal pain in March, 1953, and this was associated with nausea and vomiting. A hemogram revealed 11,100 WBC, with 70 per cent neutrophils, 28 per cent lymphocytes and 2 per cent eosinophils. Surgery was performed and the appendix removed. No post-operative data were available.

Case 3. A 27 year old white female had the appendix removed incident to other abdominal surgery in June, 1953. No additional information of value was obtained.

Case 4. A 27 year old white female was admitted to the hospital in October, 1953, complaining of pain in the right lower quadrant, nausea and vomiting. The appendix was removed. Other data on the patient were not available.

PATHOLOGY OF THE APPENDICES

Case 1. This appendix was 6 cm. long, had a smooth and glistening serosal surface and a bulbous tip. On sectioning, the lumen was found to contain a fecalith just proximal to the bulbous distention. Histologic sections revealed a thickened fibrotic subserosa with occasional infiltrated round cells and a thickened prominent zone of lymphoid tissue with large hyperplastic follicles. In addition there were within the lumen numerous trophozoites of *D. fragilis*, some with ingested blood cells, several adult pinworms, *Enterobius vermicularis*, and two ova of *T. trichiura*. Diagnosis: (a) lymphoid hyperplasia of the appendix, (b) organized peri-appendicitis and (c) fibrosis of the appendix (Fig. 1).

Case 2. This appendix was 7 cm. long, had a smooth and glistening serosa and engorged subserosal vessels. Histologic sections revealed a submucosal and a subserosal fibrosis with neutrophils in the lumen and the mucosa. In addition the lumen contained trophozoites of *D. fragilis*, some with ingested blood cells, cysts of *Endamoeba coli* and a larva of *Strongyloides stercoralis*. Diagnosis: (a) acute catarrhal appendicitis and (b) fibrosis of appendix (Fig. 2).

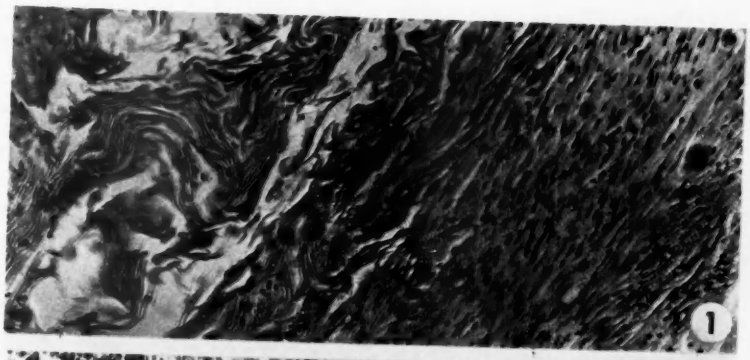
Case 3. This appendix was 6 cm. long, had a smooth and glistening serosa and

FIG. 1. *Case 1.* Fibrosis of the appendiceal wall. In Figs. 1 to 4 the mucosal area is to the left.

FIG. 2. *Case 2.* Fibrosis of the appendiceal wall

FIG. 3. *Case 3.* Fibrosis of the appendiceal wall

FIG. 4. *Case 4.* Fibrosis of the appendiceal wall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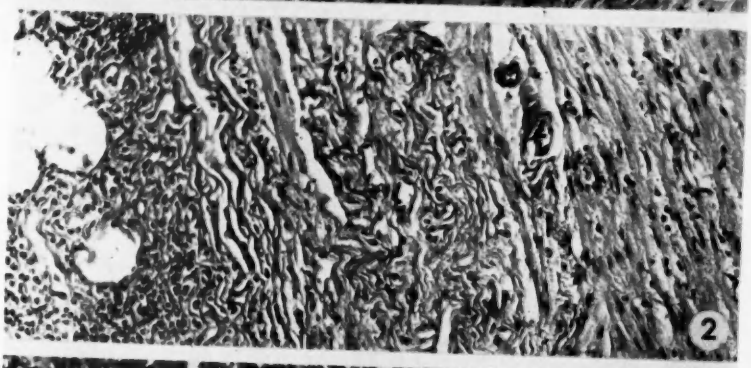
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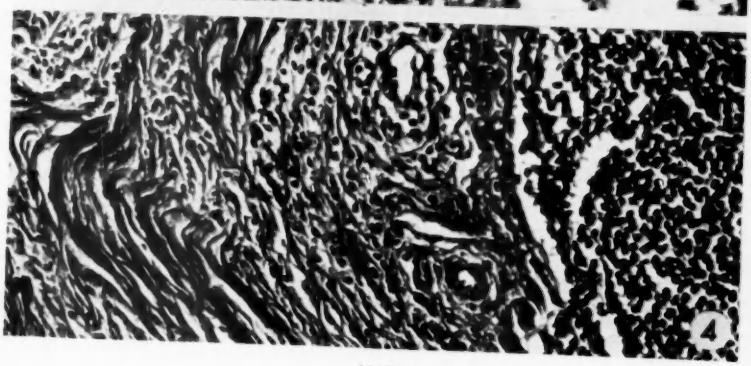
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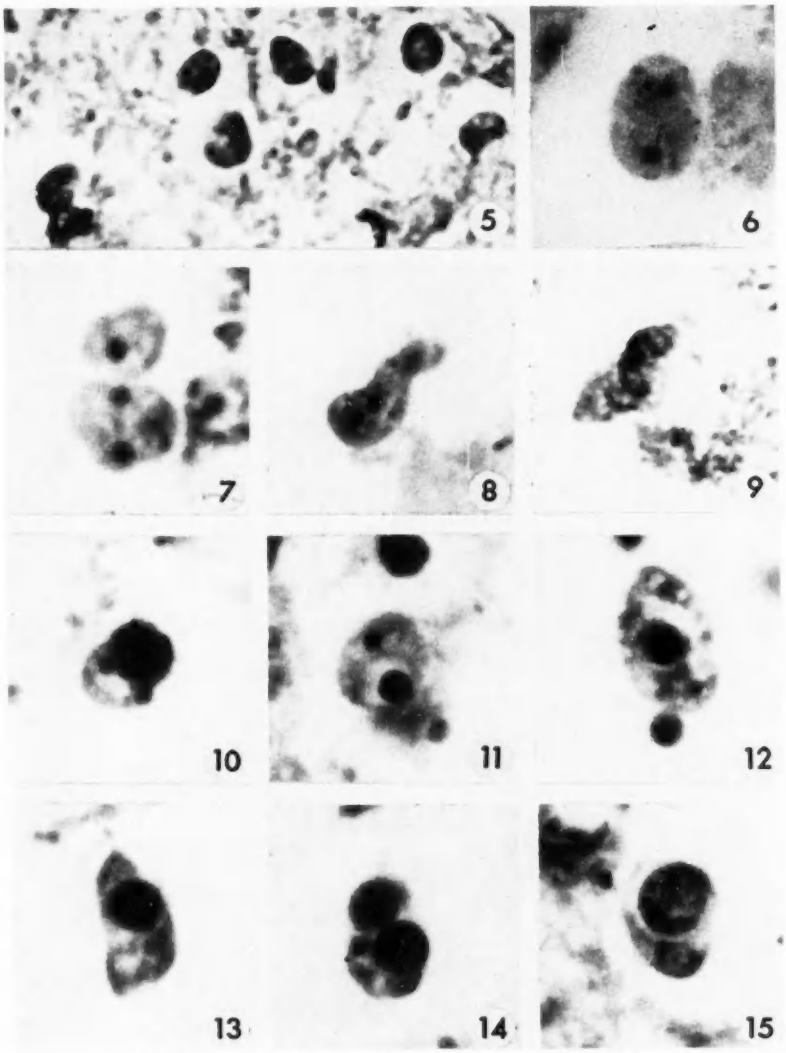

FIG. 5 magnified 800 × and Figs. 6 to 15 magnified 2000 ×

FIG. 5. Portion of the lumen of Case 1, showing seven *D. fragilis*

FIG. 6. Typical oval form

FIG. 7. Two amebae, one showing one nucleus and the other two nuclei

FIGS. 8 and 9. *D. fragilis* showing a somewhat *E. histolytica*-like progression

FIG. 10. *D. fragilis* with one ingested red blood cell and a small food vacuole. Both nuclei are shown.

FIG. 11. Partially digested red blood cell in vacuole. One nucleus only in focus

FIG. 12. A form with rather vacuolated cytoplasm and with ingested red blood cell. Only one nucleus in focus.

FIG. 13. With ingested red blood cell. Both nuclei out of focus

FIG. 14. Form showing two ingested red blood cells. One nucleus seen to left of blood cells.

Fig. 15. Ameba with ingested nucleus of leucocyte or tissue cell. Both nuclei out of focus

injected subserosal vessels. Histologic examination revealed much newly formed submucosal and subserosal fibrous tissue as well as a prominent lymphoid hyperplasia. The lumen contained trophozoites of *D. fragilis*, some with ingested blood cells. Diagnosis: (a) lymphoid hyperplasia of appendix and (b) fibrosis of appendix (Fig. 3).

Case 4. This appendix was 7 cm. long and a uniformly 6 mm. in diameter. The serosa was smooth, grayish and with prominent venules. Attached to it was 1 cm. of soft, pale, yellow mesoappendix. Histologic sections revealed a fibrotic subserosa. In the lumen there were few blood cells, a large number of trophozoites of *D. fragilis*, a few with ingested blood cells, and a single female *E. vermicularis*. Diagnosis: (a) acute peri-appendicitis and (b) fibrosis of appendix (Fig. 4).

ACTIVITY OF *DIENTAMOEBA* IN THE APPENDIX

Dientamoeba fragilis was most abundant in Case 1, rare in Case 2, present in small numbers in Case 3 and almost as abundant in Case 4 as in Case 1. The number of amebae present in ten transverse sections, from different levels, was counted in each of the four cases and the following figures obtained: Case 1, 479 *D. fragilis*; Case 2, 15; Case 3, 59; and Case 4, 336. The number of organisms showing ingested blood cells was noted in each case and the following percentages obtained: Case 1, 17 of 479, or 3.6 per cent; Case 2, 1 of 15, or 6.7 per cent; Case 3, 4 of 59, or 6.8 per cent; and Case 4, 2 of 336, or 0.6 per cent. The scarcity of amebae with ingested cells in Case 4 could be attributed to the fact that few blood cells were present in the lumen. The above figures represent minimum percentages, for many amebae, particularly in Case 1, showed food vacuoles with almost completely digested food particles, many of which may have been blood cells originally.

Most of the photographs of *D. fragilis* were made from Case 1, as amebae showing hemophagic activity were more easily found, due to the larger number of organisms present. In addition to the round and oval forms (Figs. 5 to 7), which were most common, a fair number showed a somewhat *E. histolytica*-type of progression within the lumen (Figs. 8 and 9). Numerous forms were found which had ingested from one to three red blood cells each (Figs. 10 to 14). Other amebae were observed which contained nuclei of leucocytes or of tissue cells (Fig. 15).

Approximately 350 sections from different levels of the four appendices were examined in an effort to find possible invasion of the wall of the appendix by this ameba. In no case was invasion found.

DISCUSSION

Of the 45 reported cases of infection with *Dientamoeba fragilis* described in the references already cited, which were accompanied by various symptoms, over 70 per cent had diarrhea, dysentery, abdominal pain or a combination of these symptoms. Rectal irritation, nausea with or without vomiting, fatigue and malnutrition or weight loss were reported in 11 to 20 per cent of the cases. Other symptoms reported by two to four patients each were: headache, irrita-

bility, anorexia, malaise, eczema, cutaneous lesions and fever. Seven other symptoms were recorded a single time each.

In addition to the detailed accounts of symptoms reported in the above papers, two papers compared the incidences of symptoms found in *E. histolytica* and *D. fragilis* infections. Wenrich, Stabler and Arnett (1936) stated that in their survey carriers of *D. fragilis* reported more symptoms than did carriers of *E. histolytica*. Sapero (1939) found that 43.4 per cent of the patients harboring *E. histolytica* and 27.3 per cent of those with *D. fragilis* presented complaints. In each paper a list of symptoms for *E. histolytica* was given, but none for *D. fragilis*.

Although the present cases show that *Dientamoeba* will phagocytose blood cells, as does *E. histolytica*, there is no evidence from these cases that it will actually invade tissues. On the other hand, in those appendices in which the writers have encountered other nonpathogenic amebae, such as *Endamoeba coli* and *Endolimax nana*, they have been unable to find any of these species with ingested blood cells. There is a reported case by Tyzzer and Geiman (1938) in which *E. coli* was found to phagocytose red blood cells from a bleeding papillomatous polyp of the rectum.

Hakansson (1936) considered that, in the case he reported, the symptoms observed were not the result of invasion by *D. fragilis*, but that the ameba was an irritant in the intestine. Somewhat in line with this is the fact that all four appendices revealed a marked fibrosis which could be interpreted as a response to a low grade irritation over a period of time. Inasmuch as three of the appendices showed worm infections in addition to *Dientamoeba*, the part these may have played in causing the fibrosis had to be considered. Thirteen appendices which had *E. vermicularis* as the only parasite were rechecked and only three showed fibrosis. As 77 per cent of these appendices, including one with eleven adult pinworms, failed to show fibrosis, it could be assumed that pinworms are not generally responsible for this condition. In Case 1, only two ova of *T. trichiura* were recovered from the contents and they could not be considered the cause of the fibrosis. In Case 2, a single larva of *S. stercoralis* was found in the lumen and no evidence existed that it had been in the appendiceal wall or that it had been in the lumen long, as it was still in the rhabditiform stage. Therefore, it is believed that the other parasites found in three of these appendices are unlikely causes of the fibrosis.

Dientamoeba fragilis appears to fulfill at least two of the conditions which are used to distinguish pathogenic protozoa from nonpathogenic species. One is the ingestion of blood cells and the other is the apparent production of a localized tissue reaction—fibrosis of the wall. Both conditions were found in each of the four cases of *D. fragilis* infection of the appendix. However, the pathogenicity of this species appears to be of a lower order than that of *E. histolytica* or *Balantidium coli*.

SUMMARY AND CONCLUSIONS

Dientamoeba fragilis has been encountered in four appendices and in each case some of the amebae were seen with ingested blood cells. No actual invasion of

tissue was found in any case. A marked fibrosis, which could be caused by a low grade irritant, was present in the wall of each appendix. Three of the four appendices also contained worm ova, larvae or adults, but the fibrosis could not be attributed to these. *Dientamoeba* appears as the most likely cause of this fibrosis.

Inasmuch as various symptoms have been reported in *D. fragilis* infections; as these symptoms generally disappear when the parasite is eliminated; as these amebae are shown to ingest blood cells; and as it apparently acts as an irritant and causes a localized tissue reaction, such as fibrosis, this species probably should be considered as a pathogenic species, but with a lower order of pathogenicity than that produced by *Endamoeba histolytica* or *Balantidium coli*.

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CHRONIC PNEUMONIC PLAGUE IN *MACACA MULATTA*

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It has been known since the early studies of the German Commission (Gaffkey, *et al.*, 1899) that some species of monkeys and certain human individuals were refractory to infection with the plague bacillus. Likewise, in all studies directed toward determining a lethal respiratory exposure dose, there is a tacit assumption that a host may dispose of a certain number of inhaled organisms without developing frank signs of infection. Nevertheless, the idea that preliminary pneumonic plague is uniformly fatal in the absence of specific therapy has long been current. Recoveries of human cases of pneumonic plague of primary origin are rarely reported. Thus, no recoveries from pneumonic plague were reported in the Manchurian epidemic of 1910–1911 (Hill, 1911), nor in the epidemic of 1920–1921 (Wu Lien-Teh, 1926).

Tieh *et al.* (1948) reviewed a series of 9 cases from the literature between 1925 and 1948 which were reported to have recovered. All had received some form of therapy, such as specific antiserum, sulfadiazine, bacteriophage or penicillin. Some had been vaccinated, and in certain cases diagnosis by laboratory methods had not been made. Munter's (1945) well-authenticated case had been given vaccine and had received sulfadiazine almost from onset. The three cases added by Tieh *et al.* (1948) all received sulfadiazine. Since that time, sulfadiazine and streptomycin (Huang *et al.*, 1948), chloramphenicol and Terramycin (Mercier *et al.*, 1953) have been used in the treatment of pneumonic plague with good results.

During the course of the present study, it was found that survivors from a group of monkeys (*Macaca mulatta*) exposed to infectious aerosols of *Pasteurella pestis* developed subacute or chronic lesions. It is not known whether death would eventually have ensued if the animals had not been sacrificed; however, four of the group were alive and apparently recovering 54 days after exposure to the aerosol when they were sacrificed and autopsied. Since the time interval is considerably greater than the usual survival time of animals afflicted with primary pneumonic plague in an acute form, the term "chronic pneumonic plague" is suggested.

METHODS

Exposure of monkeys. Dilutions were made of 24 hour, second passage cultures of *P. pestis* (strain 139L) in heart infusion broth (Difco). The resulting suspensions were aerosolized in a Wells-type atomizer which has been shown to produce clouds of particles having a mass mean diameter of approximately 1 micron (Goldberg, 1950). The dilution of the original culture yielded clouds having varying concentrations of organisms per liter of air. The apparatus used for conducting the cloud to the nose and the mouth of the animals was a modification (Wolochow *et al.*, 1953) of that designed by Henderson (1952).

TABLE 1

Numbers of monkeys surviving or dying following exposure to P. pestis *Strain 139L*

Trial	Dosage per Monkey (× 10,000)	Deaths	Time of Death (days after exposure)	Survivors
1	2.4	0		8
	2.8	1	13	7
	11.2	2	11,12	6
2	1.4	4	5,7,11,11	4
	4.2	4	6,7,7,11	4
	77.0	6	5,5,5,6,7,8	2

The bacterial count of clouds to which monkeys were exposed was determined by collecting samples of the aerosol in an impinger sampler (Rosebury, 1947), to which a known amount of collecting fluid had been added. One-tenth per cent XEC-151[1] in heart infusion broth, as suggested by Krueger *et al.* (1953) was added to the collecting fluid. Actual cloud concentration was determined by plating samples of dilutions of the collecting fluid and performing colony counts after appropriate incubation. Dosage per monkey was calculated from this figure (Rosebury, 1947).

Autopsy. Monkeys which did not die within 15 days of exposure to *P. pestis* clouds were considered survivors in the sense that death occurring later than this time interval would not be due to acute pneumonic plague (see Discussion). All animals were sacrificed by injection of large doses of nembutal except for four monkeys in Trial 2 which were chloroformed. Sections for histopathological study were taken from the lungs, liver, spleen, and at times from the suprarenal gland and kidney.

Bacteriology. Cultures were made by rubbing the cut surfaces of organs against the surface of tryptose blood agar medium (Trial 1). In Trial 2, fragments of organs were ground in sterile mortars with pestles and sterile Monterey sand. About 7 ml. of heart infusion broth was added to the mixture before completion of grinding. The suspension was inoculated on blood agar plates in 0.1 ml. quantities and injected into guinea pigs in 0.5 ml. quantities, either the subcutaneous or intraperitoneal routes being used.

RESULTS

Table 1 indicates that 21 of the 24 monkeys in Trial 1 survived exposure to *P. pestis* clouds. In at least two cases, however, typical mouse-virulent, phage-sensitive *P. pestis* was isolated from the organs when the survivors were sacrificed at prolonged intervals after exposure. In three other cases autopsied at 15 and 20-day intervals, organisms were isolated in great numbers. These were characteristic of *P. pestis* with respect to microscopic and colonial morphology and the rate of growth but were not sensitive to bacteriophage and were avirulent (Table 2).

[1] Dow-Corning Antifoam "A".

TABLE 2

Results of cultures and guinea pig inoculations from tissues of monkeys surviving exposure to P. pestis *Strain 139L*

Trial	Nos. of Monkeys	Sacrificed (days after exposure)	Culture	Guinea Pig Inoculation (from tissue)
1	1	15	+*	ND
	1	15	+†	ND
	2	20	+†	ND
	3	20	−	ND
	8	21–23	−	ND
	1	48	+*	ND
	3	48	−	ND
2	3	27	−	−
	2	40	+*	+
	1	40	−	−
	1	54	+‡	+
	1	54	−	?§
	2	54	−	−

ND—Not done.
* Typical phase-sensitive, guinea pig virulent strain recovered.
† Atypical strain.
‡ Phage resistant, guinea pig virulent.
§ Guinea pigs died within 24 hours after inoculation.

Upon repeating the experiment (Trial 2), a more even balance between death and survival was obtained. In all cases, a suspension obtained from the lungs, livers, and spleens of the sacrificed survivors was inoculated into guinea pigs as well as on culture media. Positive results were obtained in 3 of the 10 cases studied (Table 2).

Anatomical lesions. Marked differences in appearance were noted in the lungs of the various monkeys. However, the general picture at autopsy can be well described in terms of the following cateogories:

Category 0—*Negative.* No gross pathological change.

Category I—*Focal plague lesions.* These are about 2–3 mm. in diameter, dark red or plum-colored.They are separated from the surrounding lung tissue by a more or less marked ring of engorged pulmonary tissue. The central portion of the plague lesion histologically (Figure 1) consists of necrotic tissue in which the alveolar walls have been destroyed, although their outlines are indicated by erythrocytes. The second zone is an area of intact alveolar walls, while the third zone is an area of intense hemorrhage, congestion, and edema. In the acute form of pneumonic plague, the lesion appearing at a pleural surface is conical and sharply raised; in the chronic form, it appeared more convex and uniformly rounded.

Category II—*Consolidation.* The involved lobe is liver-like and airless, may be considerably contracted, and sinks in fixative.

Category III—*Dusky, grey, disseminated lesion.* This description applies to

FIG. 1. Focal plague lesion in monkey lung. Masses of bacilli are shown in the center of the necrotic lesion at the lower right of the picture. Polymorphonuclear leukocytes show no tendency to engulf organisms. Giemsa stain.

a delicate coloration on the surface of an otherwise normal appearing pleura. The color is distributed either in irregular streaks or across the entire surface of the affected lobe.

Category IV—*Dusky, grey, focal lesion.* This is similar to the preceding category except that the area involved is strictly limited to a circular area of 3–5 mm. diameter.

Category V—*Pleural adhesions.* These are generally very firm, requiring considerable force to tear the involved lobe away from the parietal pleura. The adhesions may occur subjacent to the pleural surfaces, to the diaphragm, to the mediastinal structures, or to all of these.

Category VI—*Pleural thickening.* This apparently results from marked thickening and coagulation of exudate on or at the lung surface, producing a play of colors varying from dusky red to fluorescent greenish red. It was seen only in those lungs studied at late stages (48–54 days).

Category VII—*Mite infestation.* Lobular lesions varying from 2 to 5 mm. in diameter protruding above the pleural surface. Appearance was that of a conical, sharply elevated papilla, white or creamy grey in color and having a tough texture.

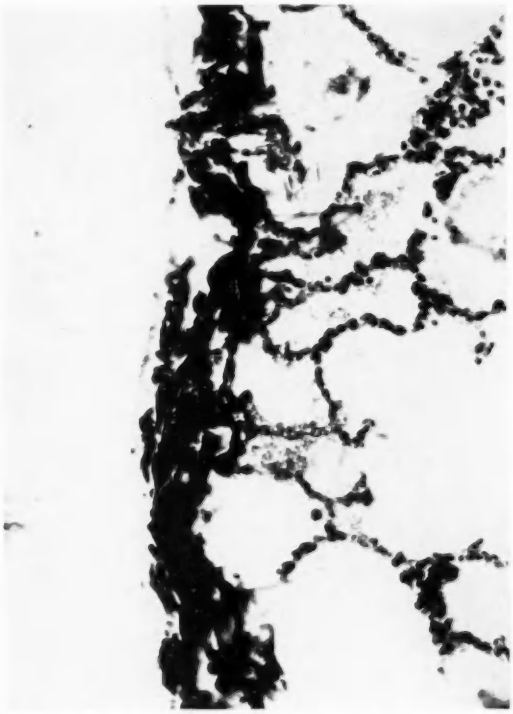

FIG. 2. Pleural thickening in chronic pneumonic plague. Compaction of tissues at the pleural surface is seen overlying the relatively normal lung structure. Such a surface presents grossly a brilliant display of colors.

The gross appearance of the lungs at autopsy reflected to a considerable extent the underlying histopathology. Thus, a normal appearing lung showed but few slight tissue changes, whereas the rather delicate, dusky grey, disseminated appearance, listed as Category III and seen most frequently in this series of autopsies, reflected in every case considerable accumulation of hemosiderin and thickening of the pleura. The animal showing consolidation of the lung was, for histopathological purposes of classification, still in the acute phase of the disease. Positive results were obtained from all tissues cultured.

Category II has been observed but once in studies in monkeys surviving exposure beyond the 15th day, although a related type of consolidation was regularly seen in monkeys dying of the acute form of the disease. In the latter, there was a marked engorgement of the alveolar capillaries, with an outpouring of polymorphonuclear leukocytes accompanied by variable numbers of macrophages into the alveolar spaces. The basic lung structure was intact. Enormous numbers of bipolar bacilli were always present in the alveolar exudate in the acute

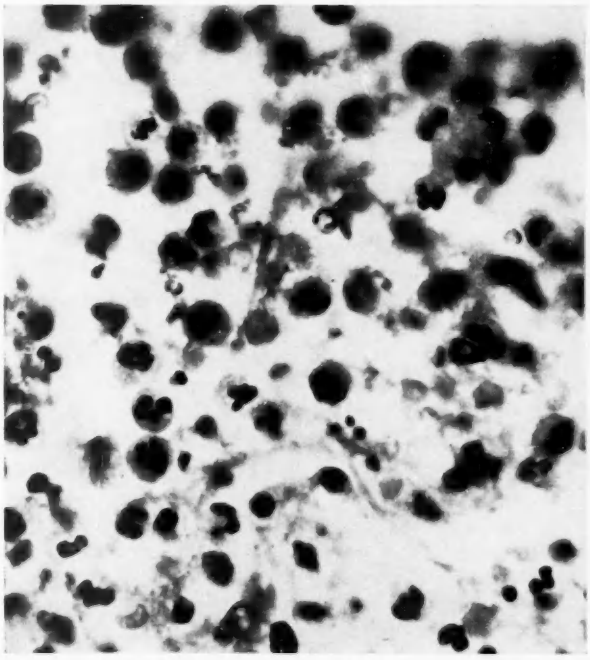

FIG. 3. Alveolar exudate in lungs, acute pneumonic plague. Organisms are seen in clumps. There is no phagocytosis. H and E stain.

type of consolidation. By contrast, the markedly contracted and consolidated lobe removed from a monkey sacrificed at 15 days after exposure displayed almost complete loss of basic lung structure due to necrosis. In addition, no organisms were visible. No differences were noted, however, in the type or quantity of alveolar exudate observed (Figure 3). Furthermore, the perivascular reaction (Figure 4), which we shall mention later on, was absent in the 15-day survivor.

Even in cases where no gross pathological change was observed, however, the animals at autopsy were not entirely negative histologically. Thus, it may be said that all of the animals autopsied showed evidence of tissue reaction. These changes ranged from severe destructive lesions in the lung, liver or spleen to slight hemorrhage and engorgement of the alveolar capillaries accompanied by small areas of infiltration by mononuclear cells. There appeared to be no regular relationship between the severity of the lesions observed and the magnitude of the dosage. There was a tendency for severe lesions to appear in monkeys sacrificed at the 15–20 day interval following exposure; the tissue reaction decreased in severity in later stages.

A striking change was seen in those animals which were sacrificed 48 days after exposure (Trial 1). The lungs appeared to have "lighted up", assuming a play

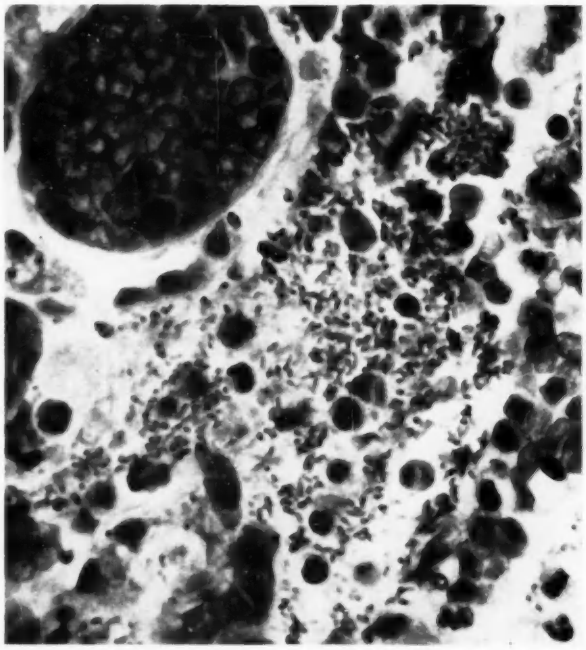

FIG. 4. Perivascular reaction in monkey lung, acute pneumonic plague. Plague bacilli are seen proliferating in the region surrounding blood vessel wall.

of colors varying from dusky red to fluorescent green. The cause of this unusual appearance lay not in the underlying parenchymal structure, which showed only moderate degrees of inflammatory reaction, but rather in the surface layer of the pleura itself, which was the site of considerable thickening and deposition of breakdown products of hemoglobin (Figure 2). In Trial 2, a similar appearance was noted in one of the animals sacrificed at 54 days, although the reaction in this case was not as intense as that noted previously.

A note of explanation is required concerning Category VII. The monkeys used for Trial 2 were of a later shipment than those used for Trial 1 and were found to be extensively infested with the monkey lung mite, *Pneumonyssus simicola* (Baker).[2] On section, the lesions produced by these arthropods were found to consist of metaplastic, granulomatous tissue surrounding a core of polymorphonuclear leukocytes, including many eosinophiles. In some cases, the core also included a circular structure which proved to be a cross-section through the body of the parasite.

Because of the great amount of granulomatous tissue present, these animals were considered unsuitable material for the histological study of chronic pneu-

[2] Identified by Dr. Deane Furman, University of California.

TABLE 3

Frequency of categories of anatomical lesions

Sacrificed (days after exposure)	Total Sacrificed	Categories							
		0	I	II	III	IV	V	VI	VI
Trial 1									
15	2	0	1	1	0	0	1	0	0
20	5	1	1	0	4	0	0	0	0
21	1	0	0	0	0	0	0	0	0
23	7	1	0	0	4	2	0	0	0
48	4	0	0	0	0	0	0	4	0
Total.....	19	2	2	1	8	2	1	4	0
Trial 2									
27	3	3	0	0	0	0	0	0	3
40	3	2	1	0	0	0	0	0	3
54	4	0	0	0	3	0	0	1	4
Total.....	10	5	1	0	3	0	0	1	10

monic plague, although there was evidence of the presence of plague lesions. For this reason, the discussion of histopathological findings is limited to the monkeys in Trial 1.

The incidence of the various categories is shown in Table 3.

DISCUSSION

Criteria of infection

The evidence that animals showing tissue reaction at prolonged intervals after exposure are infected by the plague bacillus administered by way of the respiratory route will now be considered.

First, the fact that the respiratory tracts of the animals were exposed to a virulent strain of *Pasteurella pestis* is at least *prima facie* evidence that the resulting inflammation was due to the organism. The virulence of strain 139L has been established by these and other titrations in the course of which normal monkeys have been infected (e.g., Table 1). The fatalities in these titrations have shown the typical gross pathological changes of the acute form of the disease.

Secondly, *P. pestis* was recovered from certain individuals of the chronic group (Table 2). One of these (Trial 1) was in an acute destructive phase of the disease in spite of a 15-day survival. In three other cases, the organism recovered was atypical and cannot, at present, be definitely identified as *P. pestis*. In the two cases a typical, mouse-virulent, phage-sensitive culture of *P. pestis* was recovered from monkeys which were in the late stage, indeed, of chronic infection, having survived for 48 and 54 days respectively from the date of exposure.

Thirdly, organisms resembling *P. pestis* were often seen in the tissues of mon-

keys showing chronic manifestations. When the organisms were agglutinated so that they occurred in clumps, they often stained solidly with the hematoxylin and eosin (H and E) or Giemsa stains; however, single organisms could usually be found showing the typical safety pin or bipolar-staining morphology. No attempt was made to identify involution forms as plague bacilli.

Finally, the continuing presence of hemorrhage in the lungs, of areas of focal infiltration, damage to the bronchi, engorgement, pleural thickening, and of damage to the spleen and liver, are strong evidence of infection with the agent which was responsible for the more acute disease.

Criteria of chronicity

Acute pneumonic plague in susceptible hosts follows a short and stormy course. Thus, in human beings, death usually occurs in 4 to 5 days. Robic (1937) termed a case atypical which survived for 11 days. In this laboratory, *P. pestis* strain 139L usually causes death of guinea pigs within 7 days and of mice within 6 days when administered by inhalation. Monkeys which die of acute pneumonic plague following inhalatory challenge usually die by the 11th day. Current material suggests that the period of transition from acute to subacute may occur between the 13th and 15th day following exposure. Thus, monkey #284 died on the 13th day; yet pathologically it belonged to the chronic classification. Monkey #277 survived until the 15th day, and yet showed a completely consolidated pulmonary lobe with the cultures positive for *P. pestis*, closely relating it to the acute group.

Aside from survival time, the absence of consolidation or of well-developed focal plague lesions may be taken as indicative of the chronic state. One animal in this series showed frank consolidation (Category II); however, the perivascular reaction (*vide infra*, Figure 4) was absent in this animal.

Until sufficient information is available so that one may reproduce the chronic state in monkeys at will, it is not possible to study the early phases of the chronic state by histopathological methods, since predictions cannot be made as to possible survival if the animal is not sacrificed.

Pathological characteristics of chronic pneumonic plague in monkeys

Perivascular reaction. Figures 4 and 7 illustrate the appearance of many of the pulmonary blood vessels in monkeys dying in the acute stages of pneumonic plague. Dense masses of bacilli have formed a cuff closely applied to the tunica externa; occasionally these colonies destroy the cellular layers so extensively that not even the tunica interna can be distinctly made out in stained sections. The massing of organisms in a cuff around the blood vessel was not seen in the chronic form of the disease. Instead, focal areas of infiltration consisting predominantly of mononuclear cells were observed in these sites. Such an area is shown in Figure 5, emptying through a bronchial wall. Macrophages containing breakdown products of red blood cells, chiefly hemosiderin, were present. Sometimes, single bipolar bacilli or small aggregates of these were found in the lesion. The proportion of infiltrating cells to organisms was always high and cultures were negative. Necrosis in these sites was frequently evident.

FIG. 5. Erupting bronchial lesion, acute pneumonic plague. Mononuclear phagocytes are seen traversing muscle bundle at center of picture toward epithelium at right. Giemsa stain.

Role of alveolar phagocytes in pneumonic plague

The biological significance of these differences in the perivascular reaction may be sought in the activities of the alveolar phagocytes. The path of migration of this cell has its origin in the alveolar spaces. There is much evidence (see Robertson, 1943, for a review) that its first activities as a free cell occur here, since it is said to arise from the septal cells of the alveolar walls. These cells are known to engulf particles of foreign material, including living organisms, and then to find egress from the lung parenchyma by way of pulmonary lymphatics (Forbus, 1945). The economy of the lung structure is such that lymphatic tissue within the lung substance is usually closely associated with blood vessels. If the engulfed particles are plague bacilli, the phagocyte in many cases may degenerate and discharge viable organisms into the surrounding tissue, which is now edematous and packed with free red cells, plasma, and perhaps hemoglobin, the latter well-known as a growth stimulator for *P. pestis*. This process may result in formation of colonies and damage to the vascular wall. Invasion of the bronchial walls, by way of blood vessels and lymphatic tissue with which they are well supplied, may occur simultaneously in an analogous fashion.

Secondary infiltration

The findings in chronically infected monkeys suggests that the series of events just described are either avoided or overcome. The appearance of the lesions is illustrated by Figures 5 and 8. What we see appears to be secondary infiltration of the lymphatic tissue following upon the original migration of alveolar phagocytes into this area. The cells involved are of two types; (a) the phagocytes, whose nuclei are small, dense, and compact, and (b) the lymphocytes. It should be noted that secondary infiltration of this type is not peculiar to the chronic stage of the disease; the same process is frequently seen in areas of the lungs of monkeys in the acute stage where consolidation has taken place. The process of secondary infiltration appears to have a more general distribution in monkeys in the chronic stage and may be an important factor in balancing the inflammatory reaction in favor of the host.

Inflammation of mucous membranes of the respiratory tract

The mucous membrane of the trachea, bronchi, and large bronchioles have been regarded as the primary site of lodgment of the plague bacillus (Strong, *et al.*, 1912). With inadequate clearance, it can readily be conceived that the organism could penetrate the protective epithelial covering as it does the intact, unbroken skin. However, we have seen many specimens in which focal lesions appear to have arisen from beneath the bronchial mucosa. An example is given in Figure 5, in which such a lesion was seen to erupt into the lumen of a bronchiole. In the chronic form of the disease, focal areas of infiltration of the bronchial submucosa were common (Figure 6). In addition, infiltration of the ciliated epithelium by eosinophilic leukocytes has been observed with considerable frequency. The presence of these cells is commonly taken as indicating convalescence or a tendency toward chronicity.

Hemorrhage

Hardly a section from the lungs of infected or exposed monkeys has failed to reveal some degree of rhexis of the vascular walls, either in the form of petechiae, ecchymoses, or both. In the late stage of the chronic infection, that is, in surviving monkeys sacrificed 48 days after exposure, a transition stage in the rather regular picture of hemorrhage and hemosiderin formation was observed. The red blood cells which constituted the hemorrhagic exudate were accompanied by appreciable quantities of a coarse granular material of like texture and staining reaction. A considerable amount of hemorrhage was found even at a late stage in the chronic inflammation. One occasionally encountered vascular lesions resulting in gross loss of blood even until the latest period (48 days) observed. Likewise, engorgement of the septal capillaries was a continuing feature.

Engorgement and hemorrhage may be considered a response to toxic extracts of the plague bacillus. The continuation of this lesion into the late stages of chronic and subacute inflammation may be accounted for in one case by the presence of viable bacilli in the pulmonary areas, recoverable by ordinary cultural methods. In other cases, the source of toxin must be elsewhere than in the

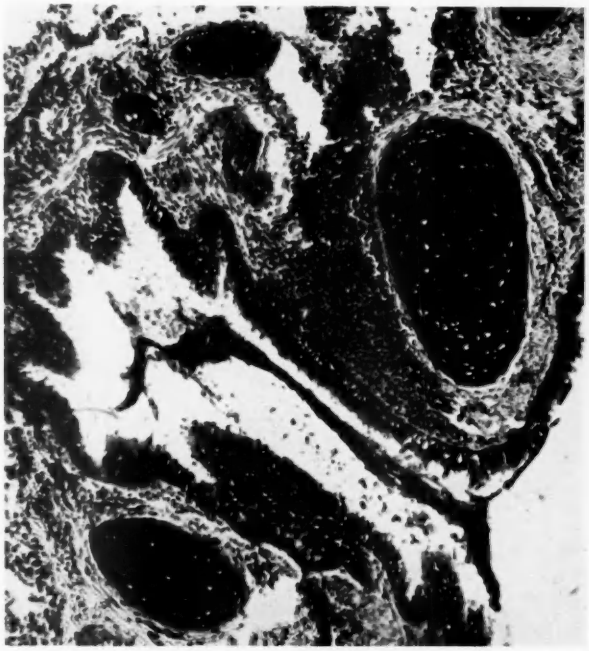

FIG. 6. Focal submucosal infiltration of bronchus, chronic pneumonic plague. Mono nuclear cells are seen infiltrating the area between the mucosa and the cartilage.

lung. Microscopically, it was extremely difficult to locate organisms even in focal areas of infiltration where the tissue reaction suggested their presence.

The spleen and liver

Even in the late stages of the disease, active foci of tissue reaction including morphologically typical plague bacilli were found in the spleen. Indeed, necrosis in this organ appeared to be emphasized as the disease process went along. At the same time, no intensification of tissue damage to the parenchymal cells of the liver or kidneys was observed; in fact, there was a gradual decrease in the degree of cloudy swelling observed in hepatic cells. Except for infection of the spleen, the impression of Forbus (1945) that changes in organs other than the lungs is of a toxic nature, was largely confirmed. In the chronic manifestation of pneumonic plague in monkeys however, there was apparently a shift from the lungs to the spleen as the site of toxin production.

Theoretical factors in the pathogenesis of the acute and chronic forms of pneumonic plague

In both acute and chronic pneumonic plague, the pulmonary tissue appeared to be the field of decisive battle. One obtained the impression that the issues

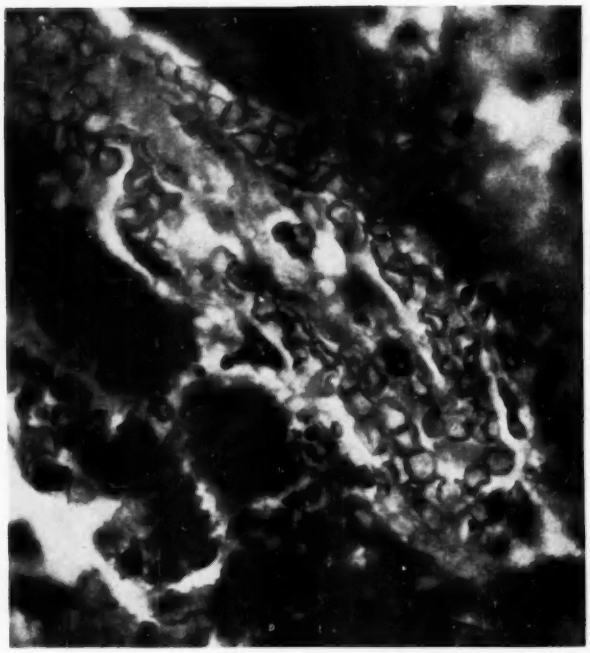

FIG. 7. Invasion of wall of pulmonary blood vessel, acute pneumonic plague. H and E stain

relating to virulence of the organism, immunity of the host, and therapeutic intervention must be decided here.

In the acute form of the disease in monkeys, which corresponds closely to primary pneumonic plague in man, the course is decided in favor of a pathogen because of its ability to proliferate. Actually, vast numbers of organisms are present before the host is overcome. The toxin of the organism is not as important in this interplay as the viable cell. The effects of toxin appear to be secondary, causing remote visceral damage which is not in itself fatal until after high concentrations of toxin have been reached.

It follows from this point of view that death would not occur under conditions where proliferation of the organism is restricted or inhibited. This seems to be the case in the monkeys suffering from chronic pneumonic plague. Whereas the organism is present in enormous numbers in the acute form (in sections observed microscopically), it is hard to find and difficult to cultivate in the chronic form. The conditions which brought about this situation in monkeys of this series are not defined. An interesting speculation is the possibility that the changes produced were toxic in nature, and that the number of viable organisms administered to the monkeys must have been too low to set up more than a transient

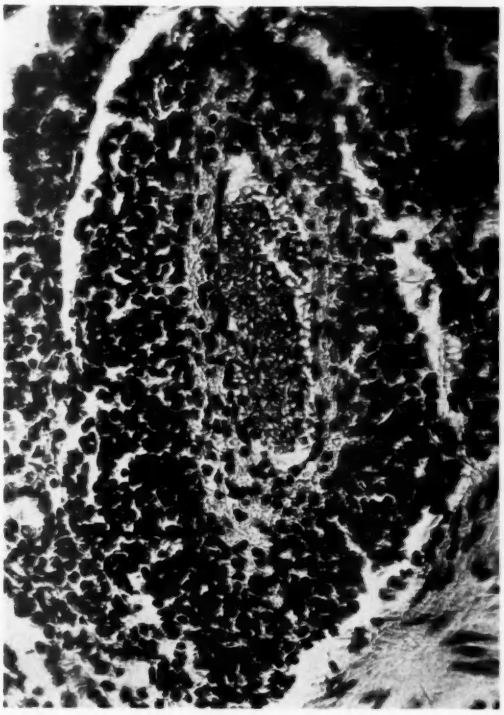

FIG. 8. Secondary perivascular infiltration, chronic pneumonic plague

infection. The possibility of such an occurrence in laboratory animals was suggested years ago by Kolle (1897).

SUMMARY

Twenty-four monkeys were exposed to *Pasteurella pestis* strain 139L administered by the inhalatory route. Dosage ranged from approximately 24,200 to approximately 112,000 organisms per monkey. Investigation of the cause of the low mortality rate (3 deaths, 21 survivors) led to the finding that survivors bore lesions which indicated a *chronic pneumonic process* in contrast to the acute character of the lesions seen in non-survivors. Virulent, phage-sensitive *P. pestis* was recovered from the survivors in two instances, one at 15 days, the other at 48 days following exposure to the infectious clouds. In additional trials, phage-sensitive *P. pestis*, still virulent for guinea pigs, was recovered from one of two animals sacrificed at 40 days. The gross and histological lesions are described.

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HISTOPLASMIN SENSITIVITY IN AMERICAN SAMOA*

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The relationship of histoplasmin sensitivity to pulmonary calcifications in tuberculin negative individuals has been demonstrated by Christie and Peterson (1945), Peterson and Christie (1948) and Palmer (1945). Also, it has been pointed out by Christie (1950, 1951) that the geographic distribution is probably worldwide and the sensitivity varies in importance from one location to another. Zeidberg *et al.* (1951) observed that the sensitivity of histoplasmin was more prevalent in damp areas than in dry ones. In Samoa the yearly rainfall exceeds two hundred inches a year, and there are no long dry seasons.

There have been two previous tuberculosis surveys on school children in American Samoa; this is the first histoplasmin survey. In these two surveys the age range was from seven to twenty years and the number of males and females was not available. The first survey in 1949 using P.P.D./N.A.H. showed 8.6 per cent of the children to be positive reactors and the second survey by the South Pacific Commission in 1951 showed 15.8 per cent positive. In the present survey, fifteen hundred and sixty-seven children between the ages of eight and twenty years were given intradermal tests of .1 cc. (1:100) histoplasmin and .1 cc. of (0.00002 mgm. P.P.D.) tuberculin. The reactions were read as positive after seventy-two hours (brochure states that the reaction becomes maximum in forty-eight to seventy-two hours) when there was an area of induration and a surrounding zone of edema measuring 0.5 cm. or more in diameter. An area of erythema without induration or edema was read as negative. All positive cases had chest x-rays on 14″ x 17″ film, and the histoplasmin positive reactors were rechecked with another intradermal test after two weeks. The results of the intradermal tests are shown in Table 1.

Three of the nine females, aged 17, 18 and 18 years, reacted to both the histoplasmin and the tuberculin tests. The remaining six females and the five males who had a positive reaction to histoplasmin did not give a reaction to the second strength tuberculin (0.005 mgm. P.P.D.).

Eleven of the fourteen cases reacting to histoplasmin showed one or more pulmonary calcifications. The three females reacting to both the histoplasmin and the tuberculin, included in the eleven, showed more than one pulmonary calcification. The three remaining cases showed only hilar adenopathy with no demonstrable peripheral lesion.

It has been pointed out by Christie (1950, 1951) that, because of the frequent moving of persons from one area to another, histoplasmosis can be expected in any part of the world. Although American Samoa is a relatively isolated island, there has been a United States Naval Base here for fifty years, and during World

* The histoplasmin was kindly supplied by Eli Lilly and Company, Indianapolis 6, Indiana.

TABLE 1

Results of intradermal tests

Age in Years	Male					Female				
	Tuberculin			Histoplasmin		Tuberculin			Histoplasmin	
	Number tested	Number pos.	% pos.	Number pos.	% pos.	Number tested	Number pos.	% pos.	Number pos.	% pos.
8–11	57	3	5.3	0	0	160	5	3.1	0	0
12–15	166	14	8.4	1	.6	214	23	10.7	1	.5
16–20	572	69	12.0	4	.7	398	85	21.4	8	2.0
Total . . .	795	86	10.8	5	.6	772	113	14.6	9	1.2

War II between fifteen and twenty thousand United States Marines from various parts of the United States were stationed here for a period of approximately three and one-half years.

SUMMARY

The results of a histoplasmin and tuberculin survey on 1567 school children in American Samoa revealed 14 positives or 0.9 per cent.

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A THEORY TO EXPLAIN THE GEOGRAPHIC VARIATIONS IN THE PREVALENCE OF HISTOPLASMIN SENSITIVITY[1, 2]

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Histoplasmosis has been reported from many countries in most parts of the world, but its true incidence is unknown. The disease is difficult to recognize clinically, laboratory diagnosis is frequently impossible, and reporting is neither uniformly required nor observed. The geographic distribution of reported cases is, therefore, a totally unreliable index of the occurrence of the etiologic agent, *Histoplasma capsulatum*. Fortunately, many studies of the prevalence of histoplasmin sensitivity have been done in various parts of the world, and, although the picture is far from complete, there is sufficient evidence to indicate that sensitivity varies geographically to a considerable degree. It may be assumed, then, that the distribution of *H. capsulatum* varies similarly (other factors, such as exposure, being equal), since infection with this agent produces allergy to histoplasmin.

H. capsulatum has been isolated many times from humans, from a variety of animals, and from several inanimate sources. Most investigators agree that the natural habitat of the fungus is soil, in which it lives freely as a saprophyte. If this is indeed so, it is logical to suggest that variations in the character and composition of soil may be the determining factor in the geographic distribution of the fungus.

The theory. Simply stated, the proffered theory is this: that red-yellow podzolic soils, because of their characteristics, provide the best soil environment for the growth of *H. capsulatum*.

If this hypothesis is valid, a significantly higher prevalence of histoplasmin sensitivity should be observed in areas where these soils predominate compared to other areas. The theory will be tested against the observed facts to be presented below.

The classification of soil. Soil is a very complex material. It is the product of many factors, "of the action of climate and living organisms upon the parent material, as conditioned by local relief" (Yearbook of Agriculture, 1938). Each of the factors is itself the product of numerous other factors. Climate is determined by temperature, humidity, precipitation, and wind direction and velocity; and it may be influenced greatly by geographic features such as mountains and

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large bodies of water. Living organisms in soil are represented by myriads of microscopic and macroscopic flora and fauna, all competing for survival. The parent material of soil frequently is the local rock which has been disintegrated by the pulverizing forces of wind and weather acting over the ages of time. The local relief or contour of the terrain influences the moisture-holding capacity of the soil and tendency to erosion. The type of vegetation growing in the soil is determined in part by the character of the soil, but in turn it influences the composition and quality of that soil. These factors and others with their many variables produce thousands of different types of soil. The types have been classified into so-called "Great Soil Groups" on the basis of common characteristics. The red-yellow podzolic soils represent one of the Groups.

Red-yellow podzolic soils are distributed in certain defined geographic areas in many parts of the world. These soils are characteristically acid and relatively shallow. They are found in warm-temperate regions where humidity is high and the average annual precipitation is between 40 and 80 inches.

The evidence. In the United States red-yellow podzolic soils are distributed over an area that corresponds quite closely with the region in which the highest prevalence of histoplasmin sensitivity has been observed (Figures 1 and 2). The correlation is by no means perfect. In Ohio, Indiana and Kansas, which have none of this soil, and in Kentucky where there is very little of it, the proportion of histoplasmin reactors is high. In Georgia, on the other hand, where more than half of the state has red-yellow podzolic soil, the prevalence of sensitivity

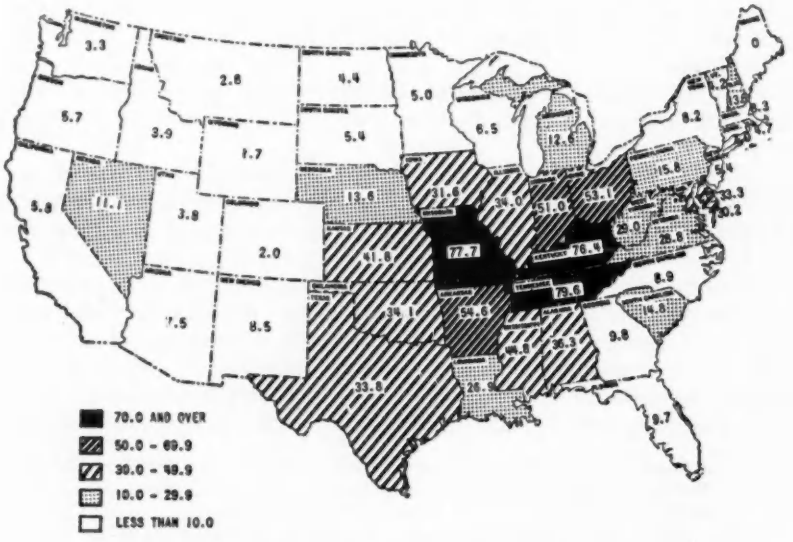

FIG. 1. Percentage of persons positive to histoplasmin by state

FIG. 2. Distribution of red-yellow podzolic soil in the United States

is low. It should be noted, however, that Emmons *et al.*, (1949) isolated *H. capsulatum* from a number of trapped wild animals in Georgia. Apparently the agent of disease is present, but why it has not infected a higher proportion of the human population remains one of the many mysteries of histoplasmosis.

Up to the present there have been few published reports of variations of histoplasmin sensitivity within a state. From the available data it is possible, in a few instances, to correlate soil groups and prevalence of histoplasmin sensitivity in a geographic area smaller than a whole state. In Tennessee, for example, the lowest prevalence is observed in the eastern part of the state (Christie *et al.*, 1946), where the practically uniform coverage of the state with red-yellow podzolic soils is interrupted by different deposits in the Appalachian and Cumberland Mountains and adjacent regions. In Illinois it has been demonstrated that sensitivity is highest in the southern part of the state (Beadenkopf *et al.*, 1953). The soil map (Figure 2) shows a wedge of red-yellow podzolic soils in the southern area of the state. These correlations cannot be accepted as proof of the soil theory advanced here, but they may be regarded as supporting evidence.

In Table 1 all states and the District of Columbia are listed according to the presence or absence of red-yellow podzolic soils. Some of the states, such as Oregon, California and Kentucky, have only a small area covered with this soil, but they have been included in the group. The number of individuals tested in each state, the number of histoplasmin reactors, and the percentage of reactors as reported in several studies (Christie *et al.*, 1946; Beadenkopf *et al.*, 1953;

TABLE 1

*Prevalence of histoplasmin sensitivity in the United States by state and by soil group**

Red-Yellow Podzolic Soils				Other Soil Groups			
State	Number tested	Positive		State	Number tested	Positive	
		Number	Per cent			Number	Per cent
Alabama	226	82	36.3	Arizona	40	3	7.5
Arkansas	174	95	54.6	Colorado	604	12	2.0
California	1,756	101	5.8	Connecticut	142	10	7.0
Florida	247	24	9.7	Delaware	24	8	33.3
Georgia	193	19	9.8	D. C.	94	31	33.0
Illinois	6,923	2,352	34.0	Idaho	77	3	3.9
Kentucky	208	159	76.4	Indiana	623	318	51.0
Louisiana	454	122	26.9	Iowa	484	153	31.6
Mississippi	221	99	44.8	Kansas	625	261	41.8
Missouri	822	639	77.7	Maine	39	0	—
North Carolina	169	15	8.9	Maryland	592	179	30.2
Oklahoma	217	74	34.1	Massachusetts	348	29	8.3
Oregon	123	7	5.7	Michigan	1,056	133	12.6
South Carolina	122	18	14.8	Minnesota	1,248	63	5.0
Tennessee	760	605	70.6	Montana	107	3	2.8
Texas	494	167	33.8	Nebraska	324	44	13.6
Virginia	191	55	28.8	Nevada	27	3	11.1
				New Hampshire	22	3	13.6
				New Jersey	407	22	5.4
				New Mexico	82	7	8.5
				New York	1,358	111	8.2
				North Dakota	159	7	4.4
				Ohio	1,447	768	53.1
				Pennsylvania	1,507	238	15.8
				Rhode Island	43	2	4.7
				South Dakota	186	10	5.4
				Utah	104	4	3.8
				Vermont	24	1	4.2
				Washington	215	7	3.3
				W. Virginia	193	56	29.0
				Wisconsin	535	35	6.5
				Wyoming	117	2	1.7
Total	13,300	4,633	34.8	Total	12,853	2,526	19.7

* References: (Christie *et al.*, 1946; Beadenkopf *et al.*, 1953; Palmer, 1946; Smith *et al.*, 1949; Loosli *et al.*, 1951).

Palmer, 1946; Smith *et al.*, 1949; Loosli *et al.*, 1951), are shown. Among 13,300 individuals tested in states with red-yellow podzolic soils 34.8 per cent reacted to histoplasmin. Only 19.7 per cent reactors were reported among 12,853 persons tested in the other states. Adjustment for population alters the percentages only slightly, to 31.7 and 17.8 respectively.

The distribution of red-yellow podzolic soils in the world is shown in Figure 3. Table 2 lists locales, according to the presence or absence of red-yellow podzolic

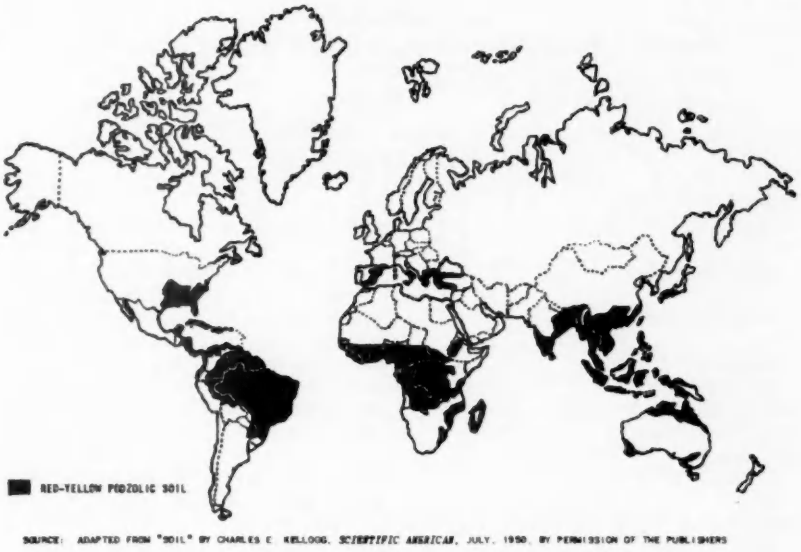
RED-YELLOW PODZOLIC SOIL
SOURCE: ADAPTED FROM "SOIL" BY CHARLES E. KELLOGG, SCIENTIFIC AMERICAN, JULY, 1950, BY PERMISSION OF THE PUBLISHERS

FIG. 3. Distribution of red-yellow podzolic soil in the world

soils, where populations have been tested for histoplasmin sensitivity. The data have been obtained from the reported literature. Some reports could not be used either because exact figures were not cited, or the residence of the individuals tested could not be defined exactly, or because no information was available concerning the kind of soil in the locale. Of 14,712 individuals tested in regions with red-yellow podzolic soils, 20.1 per cent were sensitive to histoplasmin. In other areas, only 4.6 per cent of 28,307 tested were reactors.

Very few foreign studies are available that permit a comparison between the level of histoplasmin sensitivity in a red-yellow podzolic soil locale and other areas within a single country. In Italy the proportion of positive reactors was 24.0 per cent in the Central region where there is red-yellow podzolic soil, and only 4.0 per cent in the North where a different soil is present (Mochi *et al.*, 1952.) A recent study in Burma (Tucker *et al.*, 1952) showed that 33.4 per cent of individuals tested were reactors in Rangoon where the soil is of the red-yellow podzolic group, but only 4.5 per cent of a comparable group of individuals were positive in Mandalay where the soil is of a different kind. These isolated reports throw weight on the side of the theory, but many more such correlations are needed before the balance swings conclusively to the hypothesis suggested here.

For purposes of comparison, the data cited above leave much to be desired. In the first place our present knowledge concerning the geographic distribution of the various soil groups is far from complete, and in some instances, inaccurate. Many gaps in information exist even within the United States where intensive soil studies and surveys have been going on for some time. In foreign lands,

TABLE 2

Prevalence of histoplasmin sensitivity in areas of the world by soil group

Locale	Number Tested	Positive		Reference
		Number	Per cent	
A. Red-Yellow Podzolic Soils				
Brazil				
Porto Alegre	1,025	137	13.4	(Marsiaj *et al*, 1949, 1950)
Bahia (Salvador)	229	40	17.5	(Marsiaj *et al*, 1949; Mochi *et al*, 1952)
Federal District	185	22	11.9	(Marsiaj *et al*, 1949, 1950)
Santarem	689	207	30.0	(Mochi *et al*, 1952)
Rio de Janeiro	1,574	198	12.6	(Mochi *et al*, 1952)
Burma, Rangoon & Insein	2,283	763	33.4	(Tucker *et al*, 1952)
China, Hong Kong	25	0	—	(Mochi *et al*, 1952)
Honduras				
La Lima	300	125	41.7	(Hoekenga *et al*, 1950)
Tela	422	201	47.6	(Scott, 1951)
India				
Kelur	341	0	—	(Mochi *et al*, 1952)
Vada Thorasalur	261	0	—	(Mochi *et al*, 1952)
Italy, Central	50	12	24.0	(Mochi *et al*, 1952)
Mexico				
Yucatan	150	83	55.3	(Mochi *et al*, 1952)
Abala	150	75+	50.0+	(Mochi *et al*, 1952)
Natal				
Coast	693	11	1.6	(Mochi *et al*, 1952)
Durban	1,336	79	5.9	(Mochi *et al*, 1952)
Nicaragua	137	22	16.1	(Mochi *et al*, 1952)
Panama				
Christobal	1,000	387	38.7	(Tucker, 1952)
Panama City & Colon	727	211	29.0	(Tucker *et al*, 1951)
Paraguay, Asuncion	2,106	362	17.2	(Mochi *et al*, 1952)
Philippines				
General	477	22	4.6	(Bocobo *et al*, 1950)
Manila	50	0	—	(Bocobo *et al*, 1950)
Turkey, Istanbul	502	0	—	(Mochi *et al*, 1952)
Total	14,712	2,957	20.1	
B. Other Soil Groups				
Alaska, Sitka, Wrangell	140	0	—	(Mochi *et al*, 1952)
Argentina				
Buenos Aires	437	106	24.3	(Mochi *et al*, 1952)
Villa Tortosa	58	7	12.1	(Mochi *et al*, 1952)
Cordoba	142	8	5.6	(Mochi *et al*, 1952)
Austria	1,600	5	0.3	(Kopp *et al*, 1951)
Burma, Mandalay	1,275	58	4.5	(Tucker *et al*, 1952)

TABLE 2—(*Continued*)

Locale	Number Tested	Positive		Reference
		Number	Per cent	
B. Other Soil Groups—(Continued)				
Canada				
Newfoundland	157	0	—	(Mochi *et al*, 1952)
Halifax, N. S.	310	4	1.3	(Mochi *et al*, 1952)
Winnipeg	440	23	5.2	(Mochi *et al*, 1952)
St. Agatha des Monts	270	29	10.7	(Mochi *et al*, 1952)
Antibi, East Co.	161	8	5.0	(Guy *et al*, 1949)
Beloil	75	20	26.7	(Guy *et al*, 1949)
Czechoslovakia				
Illava Dist.	386	1	0.3	(Mochi *et al*, 1952)
Dacice, Moravia	989	0	—	(Mochi *et al*, 1952)
Denmark	1,500	0	—	(Mochi *et al*, 1952)
Egypt, Mehella	380	0	—	(Mochi *et al*, 1952)
Finland, Helsinki	366	0	—	(Mochi *et al*, 1952)
France, Rhone Dept.	463	0	—	(Mochi *et al*, 1952)
Great Britain				
London	100	0	—	(Mochi *et al*, 1952)
Cardiff, Wales	144	0	—	(Mochi *et al*, 1952)
Hungary	256	0	—	(Christie, 1951)
India, Darjeeling	1,159	9	0.8	(Mochi *et al*, 1952)
Ireland, Dublin	320	0	—	(Mochi *et al*, 1952)
Italy				
Pavia	183	1	0.5	(Mochi *et al*, 1952)
Northern	303	12	4.0	(Mochi *et al*, 1952)
Mexico				
Mexico City	1,500	279	18.6	(Mochi *et al*, 1952)
Mexicali, Lower California	2,148	520	24.2	(Mochi *et al*, 1952)
Natal, Drakensburg	893	0	—	(Mochi *et al*, 1952)
Norway				
Oslo	113	0	—	(Mochi *et al*, 1952)
Bergen	1,074	50	4.7	(Böe *et al*, 1951)
Poland				
Siedlicz	293	0	—	(Mochi *et al*, 1952)
Cracow	522	1	0.2	(Mochi *et al*, 1952)
Roumania	5,000	0	—	(Mochi *et al*, 1952)
Spain, Madrid	900+	0	—	(Mochi *et al*, 1952)
Sweden	44	1	2.3	(Christie, 1951)
Switzerland				
Geneva	1,548	35	2.3	(Grasset *et al*, 1952)
Zurich	735	2	0.3	(Koller *et al*, 1948)
Union S. Africa				
Transvaal	140	11	7.9	(Mochi *et al*, 1952)
Capetown	453	3	0.7	(Jackson, 1952)
Uruguay	1,300	112	8.6	(Mochi *et al*, 1952)
Total	28,307	1,305	4.6	

particularly in Asia and Africa, very little is known about local soils in some areas. Secondly, there has been great variation in the composition of groups tested by different workers, a variety of antigens has been used, and neither dosage, technique nor criteria for reading reactions have been uniform. Furthermore, in some instances not all of the individuals tested were residents of the locale in which the tests were done. Nevertheless, the introduction of bias is unlikely since the variations were present in both the red-yellow podzolic soils group and other groups.

Discussion. Several theories have been advanced to explain the peculiar geographic distribution of histoplasmin sensitivity:

1. The average annual rainfall may be the determining factor (Furcolow *et al.*, 1951).

2. *H. capsulatum* is disseminated in the Mississippi valley by flood waters that carry the fungus in washed-out soil (Beadenkopf *et al.*, 1951).

3. There is an association between the prevalence of the fungus and the presence of large rivers running through low country in river valleys (Mochi *et al.*, 1952).

4. A combination of 1. and 3. (Tucker *et al.*, 1952).

It is not the purpose of this paper to discuss the merits of the several theories advanced. It is pertinent to point out, however, that rainfall is a part of climate, and rivers and their valleys are a part of terrain. Both climate and terrain are two of many factors that determine the character of soil in a given area.

The data presented above generally tend to support the soil theory offered here. There is no intention to imply that only the red-yellow podzolic soils can support the growth of *H. capsulatum* in nature, but rather that these soils above all others offer the best natural cultural medium for this fungus. In time, as more studies are done in parts of the world from which there is at present no information, further opportunity to test the validity of the hypothesis will be presented. If soil is indeed the determining factor in the occurrence of *H. capsulatum* in one area and not in another, it remains to be determined what particular physical, chemical and biological characteristics make it a good or poor natural habitat for the fungus.

If the soil theory is valid it could delineate possible endemic areas of histoplasmosis in various parts of the world. Since the disease is so difficult to recognize, knowledge of its geographic distribution may be helpful in alerting physicians in potential endemic areas.

SUMMARY

A soil theory is offered to explain the geographic variations in histoplasmin sensitivity in the United States and the world. It is suggested that the red-yellow podzolic soils, because of their peculiar characteristics, provide the best natural habitat for the growth of *H. capsulatum*. The theory is tested by comparing the prevalence of histoplasmin sensitivity in areas with red-yellow podzolic soils and in areas with other soils. The percentage of histoplasmin reactors is significantly higher in locales with red-yellow podzolic soils.

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AN ALCOHOL-ACID-FAST SUBSTANCE IN EGGS OF SCHISTOSOMA MANSONI

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In areas of high endemicity, eggs of *Schistosoma mansoni* have occasionally been detected in sputum smears stained by the Ziehl-Neelsen method for acid-fast bacteria. In Brazil, such findings have been reported by Silveira (1941) and by Alves Meira (1941). However, no attention has been given to the staining reaction, and we have found no record of a specific stain for eggs of *S. mansoni*. When we noted that these eggs contained an alcohol-acid-fast substance, we undertook the study reported here which involved the following successive steps:

1) Standardization of staining method for tissues and for stool smears; 2) study of specificity of staining method; 3) criteria for detection of eggs in tissues and in stool smears; 4) diagnostic value of stain in tissues and in stool smears; 5) site of staining reaction in eggs; 6) influence of chemical agents on staining reaction.

1. *Standardization of staining method for tissues and for stool smears*

In tissue sections, we employed formalin-fixed material, embedded in paraffin. The modified Ziehl-Neelsen technic for tissue sections recommended by Lillie (1948) was found to yield uniformly excellent results.

In stool sediments several variants were tested with discouraging results, among them a "wet method" of staining in centrifuge tubes. It was then found that specific staining could be obtained under the following conditions: 1) use of dried smears prepared from sediments; 2) preliminary immersion in dilute HCl; 3) prolonged differentiation in weak acid alcohol; 4) thorough counterstaining in methylene blue. When these conditions were fulfilled, satisfactory staining was obtained even with cold fuchsin. After testing a series of 128 variants in timing and concentration of the solutions, the following method was selected on the basis of specificity and convenience:

Modified Ziehl-Neelsen stain for eggs of S. mansoni in stool smears

A. Preparation of smears

1. Obtain concentrated sediments according to Hoffmann *et al.* (1934).
2. Pipette 2 to 3 drops of sediment on standard (3 x 1 inches) microscopic slide.

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3. Extend material to uniform thin film about the size of a standard (32 x 24 mm.) coverglass.
4. Dry at room temperature (one hour) or in incubator at 37° C.

B. Staining of smears

Staining then proceeds as in deparaffinized tissue sections or in Papanicolaou smears by successive transfer of slides into suitable staining dishes. Running tap water should be used for washings.

1. Immerse into 0.1 Normal HCl for one minute or longer.
2. Wash in tap water.
3. Stain in Ziehl's carbol fuchsin for ten minutes at room temperature or for two minutes at 80°C.
4. Wash in tap water.
5. Differentiate in 1% hydrochloric alcohol (concentrated HCl one part, absolute ethyl alcohol 70 parts, distilled water 29 parts) for 5 to 10 minutes (thick films should be allowed up to 10 min.).
6. Wash in tap water.
7. Counterstain in 1% carbol methylene blue for 15 to 30 seconds.
8. Wash in tap water.

Smears should be inspected after step No. 6 when they should show a faint but definite rosy tinge. If they are still red, differentiation should be prolonged for two additional minutes. Specimens may be observed while wet, or else they may be dried and covered with a drop of glycerin for observation. A panoramic objective is convenient for locating eggs; morphologic characters can be checked under low power. For permanent mounts we prefer glycerin-gelatin to resinous media, since dehydration distorts the shape of the eggs.

Large numbers of slides may be stained if the following precautions are observed: films should be uniformly thin, fuchsin should be filtered and refiltered as required, and the differentiating solution should be changed for each new batch of slides.

2. *Study of specificity of staining method*

In order to satisfy ourselves of the constancy of the stain, tissue sections and stool smears were searched for "negative" eggs. Of a series of 34 tissue blocks, 2 adjacent sections were stained with hematoxylin-eosin and with Ziehl-Neelsen stain for tissue sections, respectively, and these slides were compared. Material from 7 patients and from 12 different organs was observed. The areas were selected on the basis of those containing eggs in different phases of evolution, such as mature, dead or calcified. Two of the sections contained only residual shells and fragmented shells of eggs. No comparative study was done in stool smears, and the study was limited to a careful search for "negative" eggs in all of the 316 specimens examined.

In addition, immature eggs were obtained by collecting adult female specimens of *S. mansoni* from the portal system of experimentally infected rats. These were dried on the slide and stained as in the stool smears.

All eggs or remains of eggs found in sections and in smears stained positive.

This included all stages of development found in human material, as well as the immature eggs contained in the female worm. The stain is thus highly specific. However, variations were noted in the intensity of the stain. These were more marked in tissue sections than in stool smears. They could not be correlated with any other variable, such as developmental stage or degree of tissue reaction, and we did not measure them quantitatively. Further work is needed before it can be decided whether these variations are due to technical factors or to actual differences in the amount of positive substance in individual eggs.

3. *Criteria for detection of eggs in tissues and in stool smears*

In tissue sections it was found that eggs and fragments of eggs were stained to the exclusion of any other element, save for acid-fast bacteria. Whole eggs were identified with ease and, in addition, empty shells and fragments of shells engulfed in giant cells and buried in tissue scars were visualized where one could not see them with hematoxylin-eosin. Besides positive staining, the shape of these fragments, their degree of refringence, and the surrounding tissue reaction were found to be sufficient criteria for diagnosis.

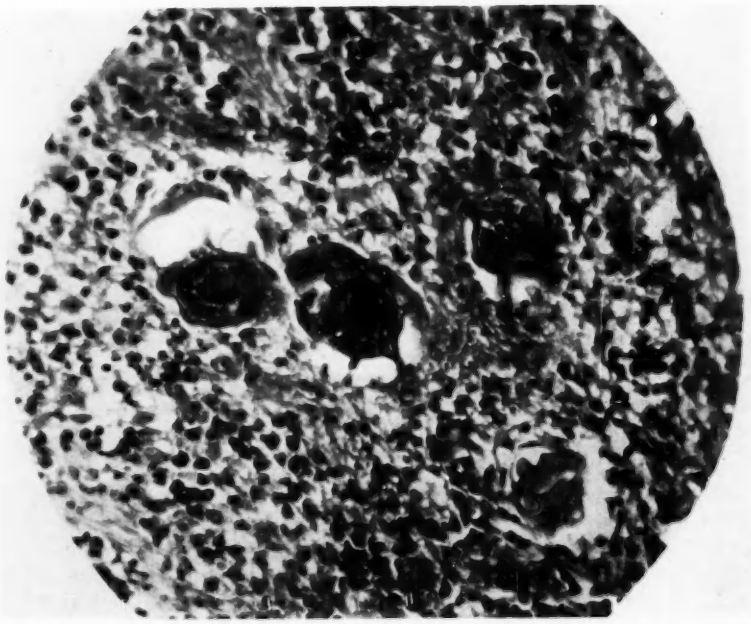

FIG. 1. Cluster of eggs of *Schistosoma mansoni*, surrounded by inflammatory tissue, from a rectal polyp. Hematoxylin-eosin, magnified 360 times. This figure is included for comparison with results of acid fast technic. Note refringence of capsule of eggs, details of miracidium and of inflammatory cells. Several giant cells are observed. Wrinkling and shrinkage of eggs in tissue sections is an unavoidable artifact.

In stool smears the results are as follows: background blue, parasitic eggs colorless or pigmented, according to species, whole eggs of *S. mansoni* brilliant red, and empty shells faintly red. In addition, a few other particles appear consistently positive, namely: 1) hair-shaped vegetable fiber remnants ("vegetable hairs"); 2) an oval-shaped vegetable remnant of unknown nature, which stained intensely, but was both larger and rounder than the eggs; 3) bean-shaped eggs of *Heterodera radicicola*, a non-parasitic helminth, the eggs of which are occasionally found in stool examinations in Brazil; 4) undigested fragments of Tyroglyphus, a small innocuous mite occasionally found in the stools, and finally, 5) some of the larger amorphous residues conserve a reddish stain in their center. None of these particles are likely to be confused with eggs and none appear in large numbers except occasionally the "vegetable hairs".

However, a double criterion was used for identification of eggs, namely, 1) positive stain; 2) presence of lateral spine. Slides were labeled positive only when both criteria were fulfilled. Otherwise they were labeled negative. In one instance, a positive egg morphologically identical with that of *S. mansoni* was seen, but the lateral spine was not detected. This slide was labeled doubtful, but on re-examination a positive result was obtained.

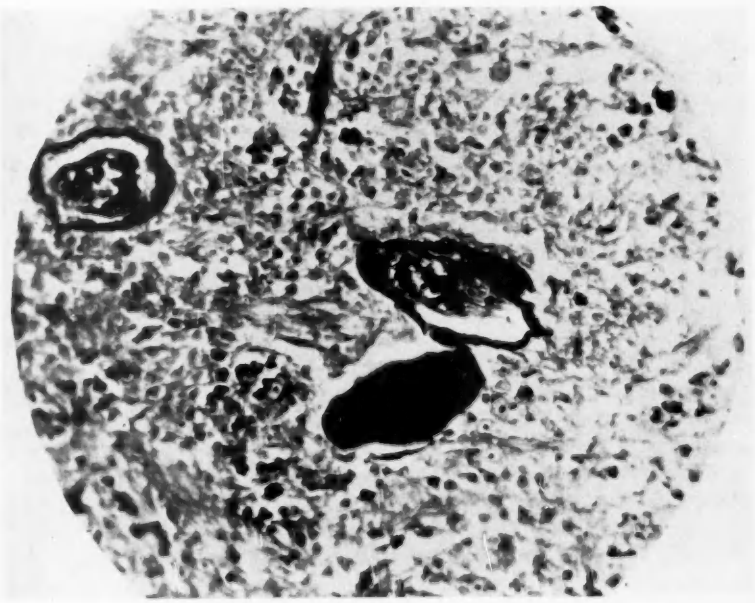

FIG. 2. Similar cluster stained by Ziehl-Neelsen technic. Same magnification. Note sharp limitation and staining of capsule and of irregular masses within the miracidium. Inflammatory cells are palely stained by methylene blue counterstain.

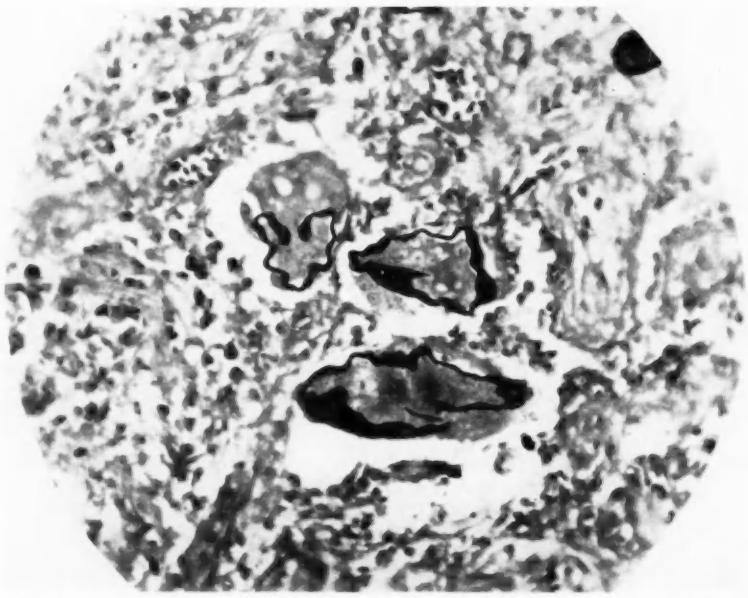

FIG. 3. Cluster of residual shells of eggs. Ziehl-Neelsen technic, same magnification. Note easy identification of these bodies, otherwise detectable only through their refringence.

4. *Diagnostic value of stain in tissues and in stool smears*

Material in tissue sections does not lend itself to useful statistical analysis. The stain, however, has been used by us as a routine procedure, and it has demonstrated its value in many instances. In three cases it has permitted the diagnosis of *S. mansoni*, even though eggs had not been demonstrated beyond doubt by routine methods. In a patient parasitologically cured of *S. mansoni* infection we were able to locate residues and fragments of eggs in the liver, which was the seat of an advanced fibrosis. In two more instances, refringent bodies of uncharacteristic appearance were shown to be rests of eggs, one within arterial lesions in a lung and another embedded between areas of tuberculous caseation in a mesenteric lymph node.

In stool smears we tested the value of the stain against that of direct examination in 316 specimens. This material was divided into two groups as follows:

Group A comprised 166 consecutive samples from our Laboratory of Clinical Pathology. Sediments were prepared according to Hoffmann *et al.* (1934). Part of the sediment was observed directly under the coverglass and simultaneously one smear of each specimen was prepared for special staining. For direct observation up to three slides were searched in negative cases.

Group B comprised 150 consecutive specimens from the Parasitological Labo-

FIG. 4. Stool smear. Modified Ziehl-Neelsen technic. Same magnification. Lateral spine protrudes slightly from the tip of the egg. The miracidium is more strongly positive than the shell. In the original, the egg is dark red, background varying shades of blue.

ratory of the "Instituto de Saude Publica".[3] Sedimentation proceeded as above, but three consecutive direct examinations were done at the Institute after 2½ hours, 6 hours and 24 hours respectively. After the first observation smears were prepared and brought to our laboratory for special staining. The results and percentages obtained are summarized in Table 1.

For further comparison, the total incidence of stool positivity for *S. mansoni*, both in our Hospital and in the Instituto de Saude Publica, as determined from a larger number of samples is given in Table 2.

It is seen that the highest incidence of positivity is secured by the special stain. The gain in percentage of positivity was highest in relation to the total figure of the Institute, next highest in relation to our total series. The difference was less marked with respect to the simultaneously run control series, and it was lowest in relation to the control series run by the Institute.

Differences in incidence depend, besides the method employed, upon the care which is given to individual examinations (Vianna Martins, 1937). It was

[3] We wish to thank Dr. Octavio Mangabeira Filho, Director of the Instituto de Saude Publica, and Dr. A. de Souza Lopes, Chief of the Section of Parasitology, for their kind collaboration in this study.

TABLE 1

	Examined, Number	Pos. on Direct Exam.		Pos. on Special Stain		Increase, Percentage
		Number	Percentage	Number	Percentage	
Group A	166	40	24.1%	77	46.4%	22.3%
Group B	150	49	32.6%	65	43.3%	10.7%
Total	316	89	28.1%	142	44.9%	16.8%

TABLE 2

	Period of Time	No. of Specimens	No. of Positives	% Pos.
Hospital das Clinicas	Jan. 1–Oct. 31, 1951	5004	851	17.0%
Instituto de Saude Publica	Sep. 1–Oct. 31, 1951	776	95	12.2%
Special stain series	Nov. 15–Nov. 22, 1951	316	89	44.9%

learned that control series B was done with more than ordinary care at the Institute, and thus represents the limit of precision to which simple inspection can go.

As to the special stain series, such a maximum was not obtained, since only one slide was examined of each specimen. The mean time for examination, as determined by stop watch, was 4 min., 16 sec., although actual time spent varied widely from intensely positive to negative specimens. The incidence of positives may be further improved by examining more than one slide, and by giving each slide a thorough scanning.

5. *Site of staining reaction in eggs*

A remarkable difference in this regard was noted between tissue sections and stool smears. In tissue sections, the shell of the eggs stained constantly, the miracidium either did not stain or it appeared with a few irregular positive spots. In stool smears, empty shells also stained constantly with an intensity comparable to that found in tissue sections. In intact eggs the miracidium stained brilliantly. In broken eggs the extruded miracidium either did not stain or it appeared with a few irregular positive spots.

In view of these differences, the following simple test was performed. Intact eggs in stained stool smears were centered under the microscope and a drop of 0.1 N HCl was deposited over them. No change was noted in the staining of the miracidium within 5 minutes of observation. By means of a fine needle, the eggs were then punctured under microscopic control and an almost instantaneous decoloration ensued. The same experiment was performed, substituting a drop of tap water for the acid. At the moment of puncture the miracidium appeared brilliantly red. However, it soon began to lose color and after 5 minutes only a rosy tinge was left.

The following conclusions were drawn from these observations: (1) the shell is weakly positive under all circumstances; (2) the miracidium is strongly positive in eggs with intact shells but is negative when the shell is not intact.

It can be assumed that acid-fast substances are present in both the shell and miracidium. But, whereas the capsular substance is eminently stable, the miracidial substance is eminently diffusible. For purpose of convenience, we named these substances provisionally Substance C (capsular substance) and Substance M (miracidial substance). As to the chemical nature and reciprocal relations of these substances, no conclusion can be drawn at this time.

6. *Influence of chemical substances on staining reaction*

A group of experiments was performed to determine whether the staining reaction could be abolished by previous chemical treatment, with the view of comparing these data with those already known about other acid-fast materials. Fragments of an appendix containing a large number of eggs of *S. mansoni* were embedded in paraffin, cut in sections 6 micra thick, deparaffinized, and immersed in diverse chemical substances as specified in Table 3. Three sections and one control were used in each experiment. They were then washed in tap water and stained by the modified Ziehl-Neelsen technic, with special care to avoid variations in timing and concentration of the solutions. They were mounted in balsam and checked against the control section, untreated and stained by the same method. Since experimental conditions were not sufficiently standardized to be submitted to quantitative analysis, only a qualitative criterion was used for labelling the results, namely:

1. Negative (−), when the stain was undiminished or slightly diminished.
2. Positive (+), when the stain was removed or nearly so.

TABLE 3

No.	Reagent	Concentration	Time	Temperature	Result
1	HCl	22%	10′		—
2	HCl	22%	60′		—
3	HCl	22%	24 hrs.		+
4	HCl	0.1%	24 hrs.		—
5	NaOH	10%	10′		—
6	NaOH	0.1%	30′	Room	—
7	Ether	100%	24 hrs.	temperature	—
8	Chloroform	100%	24 hrs.		—
9	Acetone	100%	24 hrs.		—
10	Benzol	100%	24 hrs.		—
11	Petroleum ether	100%	24 hrs.		—
12	"Solvanal"*	1%	24 hrs.		—
13	Acetone	100%	24 hrs.	42° C.	—
14	Benzol	100%	24 hrs.		—

* Trademark of a laboratory detergent.

Since alkali macerated the sections, three additional experiments were undertaken. Fragments of intestinal mucosa with numerous eggs were heated to 100°C. in 0.4% NaOH for 1, 2 and 5 min., respectively. Smears were then prepared and stained as above. The results were negative.

A positive result was obtained only in Experiment No. 3. It was found that within the limits of the experimental conditions observed, the staining is removed only after 24 hours in concentrated HCl. It is not removed by weak HCl, weak and strong NaOH, lipid solvents or detergents. Since the experiments were done on tissue sections, they refer exclusively to Substance C (capsular substance).

DISCUSSION

We believe that the following conclusions are warranted: (1) eggs of *S. mansoni* constantly display, both in tissues and in the stools, an alcohol-acid-fast substance; (2) the staining of this substance is a valuable diagnostic aid for detecting eggs in tissues; (3) a modification of the Ziehl-Neelsen stain, developed for stool smears, improves the percentage of detection of eggs, and should be tested as a routine diagnostic procedure; (4) there are two varieties of alcohol-acid-fast material, one localized in the capsule and showing a high degree of stability (Substance C), another localized in the miracidium, the demonstration of which depends on the integrity of the capsule (Substance M); 5) preliminary experiments indicate that Substance C is a highly inert chemical body, which resists drastic treatment without losing its staining ability; 6) this inertness is further shown by its permanence within the tissues, even after the egg has become partially destroyed.

The implications of these findings are both practical and theoretical. As to practical applications, little discussion seems necessary. Investigation of the method here described, on a larger scale, will show how far it may improve the present-day methods of diagnosis and where it may fall short of expectations. It should be emphasized that one cannot expect all cases of *S. mansoni* to be detected by stool examination, regardless of the method employed. It has been shown (Faust *et al.*, 1934) that eggs may be retained in large numbers in the tissues in patients in whom they are passing into the stools in very small numbers. In such cases, rectal biopsy with direct examination, as recommended by Ottolina and Atencio (1943), has proven to be of considerable value. However, this method also has its limitations and for certain purposes, such as large scale screening of populations, we would recommend the special stain as the more practical solution.

If the eggs of other species of *Schistosoma* harbor similar alcohol-acid-fast substances, the applicability of our findings will become correspondingly enlarged, and this possibility should be investigated.

As to the theoretical implications, only a few statements can be made on the basis of the data so far available. Acid-fastness has been found in so many widely divergent substances of plant and animal origin that it conveys no microchemical information on the structure and properties of the acid-fast substance.

Among the pathogenic organisms, acid-fastness is found in diverse members of the genera *Actinomyces*, *Nocardia* and *Mycobacterium* (Bergey, 1948). Dubos (1949) has found it in the egg of *Taenia*.

Anderson (1939) has isolated and described mycolic acid, the acid-fast substance of Mycobacteriaceae, which is a complex bacterial wax of high chemical inertness, extremely resistant to saponification. However, the acid-fastness of *Mycobacterium tuberculosis* can be abolished within half an hour by hydrochloric acid and by ether. Another substance isolated from the acetone fraction of tubercle bacilli has been named phthioic acid (Anderson, 1939) and has been shown to produce a histiocytic granuloma of tuberculoid aspect in the unsensitized guinea pig (Sabin).

Substance C, the stable capsular substance of the ovum of *S. mansoni* also shows considerable chemical inertness. Acid-fastness resists alkali, lipid extractors, detergents, and weak acid for many hours. Even after 24 hours in strong acid, it is not completely lost; in this respect it resists better than mycolic acid. It is found in old granulomatous lesions and in scars withstanding tissue enzymes and macrophages. There is no proof that any chemical resemblance exists between Substance C and mycolic acid, and even a similarity would appear to be unlikely. However, one may ask whether Substance C possesses, in its own right, a significance in the pathogenesis of schistosomiasis, or whether it indicates the presence of other substances responsible for tissue reaction. This hypothesis cannot be dismissed easily.

The "elementary lesion" of *S. mansoni* has been described as a granuloma with tuberculoid features harboring an egg in its center (Jaffé, 1948; Koppisch, 1941). This granuloma has been produced in the lung of unsensitized dogs by injection of purified eggs into a cervical vein (Sorour, 1929). There is abundant clinical and experimental evidence to the effect that other non-granulomatous lesions exist, and many authors believe that the more important features of the disease are due to the biological activity of the adult trematode (Rodrigues da Silva, 1949). However, since it has been shown that the egg contains an acid-fast substance, further attention should be given to the chemistry and the biological properties of the egg.

The obvious next step in this direction is to obtain eggs in a pure and essentially unmodified state. Once accessible, eggs may then be submitted to chemical analysis with methods parallel to those of Anderson (1940). They may be tested for special biological properties in animal experiments and extracts may be used for immunological studies. Considerable work will have to be completed before the biological significance of the acid-fast substance of eggs of *S. mansoni* is fully understood.

SUMMARY

A specific alcohol-acid-fast substance in eggs of *Schistosoma mansoni* is described, which is regularly demonstrable in tissues and in the stools. Specific staining improves detection of eggs and of rests of eggs in tissue sections. A modification of the Ziehl-Neelsen stain for stool smears is described. This modi-

fication is shown to yield a higher percentage of positivity than direct examination, and is recommended for routine use.

Two varieties of acid-fast material are shown to exist in the egg, one of them in the capsule (Substance C), and the other in the miracidium (Substance M). The first of these stains constantly; the latter only when the capsule is intact. Substance C is shown to be resistant to drastic chemical treatments and to persist in the old lesions within the tissues even after the egg has become fragmented.

Note: We want to thank Dr. Enrique Koppisch of the University of Puerto Rico, School of Medicine for the revision of the manuscript and for many valuable suggestions.

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POLYVALENT ANTIVENIN IN THE TREATMENT OF EXPERIMENTAL SNAKE VENOM POISONING

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Although antivenin or immune serum produced against snake venom has been used in the treatment of snakebite since 1895, there is still widely divergent opinion regarding its worth. Antivenin became generally available in the United States about 1926, and soon thereafter Hutchinson (1929) reported that the death rate in a series of 433 serum treated cases was 3 per cent in contrast to a death rate of 10.8 per cent in a series of 607 cases treated without serum. No comparable series of cases has since been reported for the United States, but it is noteworthy that Jackson (1929) reported no fatalities in a series of 17 cases treated by incision and suction alone, and 2 fatalities in a series of 23 cases treated by serum alone or a combination of serum, incision and suction. It is difficult, however, to assess the worth of antivenin from clinical reports of its use. No two cases of snakebite present quite the same problem. The quantity and toxicity of the venom, the depth and location of the bite, the ability of the body's defenses to respond, and the time lapse between injury and treatment are all subject to wide variation which may influence the outcome. Antivenin is rarely the sole therapy but is regularly combined with such measures as incision and suction, blood transfusion, intravenous fluids, antibiotics, and various analgesic and supportive drugs. While such combined therapy has produced a gratifying reduction in snakebite fatalities, the relative value of individual components becomes difficult to determine. Finally, snakebite is an unusual injury and few physicians have the opportunity to treat or see a number of comparable cases.

Snake venom poisoning, in theory, is an excellent situation for the employment of specific serotherapy. The snake-bitten individual receives the entire amount of toxin in a single dose at an easily established time. Even under the most unfavorable circumstances, this amount is probably not more than ten or twelve lethal doses, and in the vast majority of cases is much less. Moreover, a very appreciable quantity of venom may be removed from the tissues by mechanical means. The neutralization of the remaining venom seems a rather modest assignment for an antitoxic serum if one considers that a therapeutic dose of 5,000 units of good quality diphtheria antitoxin is able to neutralize 300,000 to 500,000 guinea pig LD_{50} of toxin and prevent disease in an actively infected host.

It is neither practical nor desirable to produce monovalent antivenins against the venom of each species of snake. There are currently recognized 16 species of rattlesnakes in the United States, most of which are divided into 2 to 6 subspecies. To produce specific sera for all these forms as well as the copperhead, water moccasin, and coral snakes would scarcely be economically feasible even

if venoms of the rarer species could be obtained in quantity sufficient to immunize animals for serum production. Furthermore, the reptile responsible for a given case of snakebite may not be available or may not be correctly identified. It is the usual practice to produce polyvalent antisera against the venoms of the most abundant and dangerous snakes of an area and against venoms which, because of their antigenic relationships, afford the greatest degree of paraspecific action. The existence of group antigens in snake venoms has been demonstrated by a number of workers. These antigens are variable in amount and their occurrence or absence does not always follow accepted phylogenetic relationships between species of snakes. According to the data of Andrews and Pollard (1953), the North American polyvalent antivenin, presumably the same material used in the experiments reported here, is prepared by the immunization of horses with a mixture consisting of 33.33 per cent tropical rattlesnake (*Crotalus terrificus*) venom and 16.67 per cent each of the venoms of Maximilian's pit viper (*Bothrops neuweidii*), the fer-de-lance (*Bothrops atrox*), the western diamondback rattlesnake (*Crotalus atrox*) and the eastern diamondback rattlesnake (*Crotalus adamanteus*). Since the first three species are neotropical and do not occur in the United States, they are evidently included because of the high degree of paraspecific protection afforded by antisera produced against them.

EXPERIMENTAL

The experiments here reported were undertaken as part of a general investigation of the comparative toxicity of North American snake venoms and their antigenic relationships. Venoms from the following North American pit vipers were used:

(1) The copperhead (*Agkistrodon contortrix mokeson*): this is a rather small snake with a wide range in the eastern United States. It accounts for a large number of snakebite cases but mortality is very low.

(2) The cottonmouth or water moccasin (*Agkistrodon piscivorus spp.*): this species accounts for the majority of snakebite accidents in Louisiana (Swartzwelder, 1950), and is important in other southern lowland states.

(3) The swamp rattlesnake or massasauga (*Sistrurus c. catenatus*): included primarily as a representative of its genus which is quite distinct from the larger rattlesnakes. Not a very dangerous species.

(4) The timber rattlesnake (*Crotalus h. horridus*): the large rattlesnake of the northeastern and central United States.

(5) The eastern diamondback rattlesnake *Crotalus adamanteus*: an extremely large and very dangerous rattlesnake of the southeastern United States.

(6) The western diamondback rattlesnake (*Crotalus atrox*): nearly as large as the preceding species and probably more aggressive. Generally considered responsible for the greatest number of fatal snakebites in the United States.

(7) The San Diego rattlesnake (*Crotalus viridis helleri*): a representative of a subspecies complex with a very wide range in western North America. Provided the most highly toxic venom tested.

(8) The horned rattlesnake or sidewinder (*Crotalus cerastes laterorepens*):

TABLE 1

Toxicity of snake venoms as determined by subcutaneous injection of mice

Type of Venom	LD_{50} in Milligrams per Kilo
Massasauga rattlesnake (*Sistrurus c. catenatus*)	5.25
Sidewinder or horned rattlesnake (*Crotalus cerastes laterorepens*)	5.50
San Diego rattlesnake (*Crotalus viridis helleri*)	3.56
Timber rattlesnake (*Crotalus h. horridus*)	9.15
Eastern diamondback rattlesnake (*Crotalus adamanteus*)	14.55
Western diamondback rattlesnake (*Crotalus atrox*)	19.25
Copperhead (*Agkistrodon contortrix mokeson*)	25.65
Cottonmouth or water moccasin (*Agkistrodon piscivorus*)	25.85

included primarily because it is a highly specialized rattlesnake of uncertain phylogenetic position. Venom highly toxic.

Most of the venom samples were extracted in the writer's laboratory and dried immediately thereafter. The toxicity of the venoms was determined by subcutaneous injection of white mice in the manner described previously (Minton, 1953). The LD_{50} for each of the venoms used is given in Table 1. Before use the dried venom was dissolved in distilled water to a concentration of 2 milligrams per milliliter.

Female Swiss mice weighing 15 to 25 grams were used. Animals were weighed individually and the venom dose adjusted accordingly. Venom injections were made under the loose skin of the shoulder area. A dose of 2 LD_{50} of venom was used in all experiments reported. This dose was selected because it gave almost 100 per cent mortality in controls yet was sufficiently small that neutralization of as little as one LD_{50} might be expected to produce a significant increase in survival Moreover, it is my opinion that this dose roughly approximates the proportionate amount of venom injected in most potentially fatal cases of snakebite in man.

The antiserum used was "Antivenin (Nearctic Crotalidae) polyvalent" manufactured by Wyeth Incorporated of Philadelphia. The lyophilized material was purchased on the open market and restored to liquid state according to directions supplied. The reconstituted antiserum was stored at −40°C. when not in use. The dose administered to each mouse was 0.1 ml. (approximately 5 ml. per kilo of body weight). In one experiment, antivenin was administered 15 minutes before injection of the venom, and in two experiments antivenin was injected 10 to 15 minutes after injection of venom. Antivenin was injected intraperitoneally in two experiments, and in one experiment it was injected into the subcutaneous tissue at the site of venom injection. Control mice received injections of normal horse serum at the same time intervals and in the same dosage as the antivenin. All deaths of mice within 72 hours following injection were ascribed to the effect of snake venom. Probability (P) values, were calculated using the procedure of chi-square with Yate's correction for small samples. Results are summarized in Table 2.

TABLE 2

Effect of 0.1 ml. of antivenin upon animals receiving 2 LD/50 of snake venom

Type of Venom	Results (Animals surviving/Animals injected) P values, where indicated, in parentheses			
	Antivenin intraperitoneally 15′ before venom	Antivenin intraperitoneally 15′ after venom	Antivenin subcutaneously 15′ after venom	No antivenin
Massasauga rattlesnake, *Sistrurus c. catenatus*	6/11 (P = .05–.02)	3/12 (P = .25–.20)	0/6	0/12
Sidewinder or horned rattlesnake, *Crotalus cerastes*	9/12 (P = .01–.001)	11/13 (P < .001)	5/6 (P = .05–.02)	2/16
San Diego rattlesnake, *Crotalus viridis helleri*	7/11 (P = .01–.001)	10/12 (P < .001)	5/11 (P = .05–.02)	0/12
Timber rattlesnake, *Crotalus h. horridus*	3/6 (P = .10–.05)	5/14 (P = .02–.05)	1/12	0/14
Eastern diamondback rattlesnake, *Crotalus adamanteus*	2/12	3/12 (P = .30–.20)	1/10	1/18
Western diamondback rattlesnake, *Crotalus atrox*	1/12	0/12	0/7	0/12
Copperhead, *Agkistrodon contortrix mokeson*	0/6	0/12	0/6	0/12
Cottonmouth or water moccasin, *Agkistrodon piscivorus*	0/6	0/12	0/12	0/18

DISCUSSION

Statistically significant protection (P = 0.05 or less) was obtained in the experiments using the venoms of the San Diego rattlesnake (*Crotalus viridis helleri*) and the horned rattlesnake (*Crotalus cerastes*). A less significant but probably genuine effect was noted against the venom of the timber rattlesnake (*Crotalus horridus*), and a questionable effect against the venom of the massasauga (*Sistrurus catenatus*). The serum treated survivors in the groups receiving diamondback rattlesnake venoms (*Crotalus atrox*, *C. adamanteus*) are too few to be significant, and no treated animals survived in the groups receiving copperhead or water moccasin (*Agkistrodon*) venoms. It is noteworthy that none of the venoms against which protection was demonstrated are used in the production of the polyvalent antivenin. Similar paraspecific activity was reported by Githens and Butz (1929) who found that a globulin concentrate from the plasma of horses immunized against *Crotalus atrox* venom was more effective against the venoms of *Crotalus adamanteus*, *C. horridus*, and *C. oreganus* (= *C. v. helleri* in part) than against *atrox* venom. Githens and Wolff (1939) reported that a polyvalent antivenin produced by immunization of horses with a mixture of 70 per cent *Crotalus atrox* venom and lesser amounts of four other rattlesnake venoms was more effective in neutralizing the venoms of *Crotalus v. viridis*, than it was against the venom of any snake in the mixture. The venoms of *C. v. oreganus*, *C. molossus*, and *C. mitchelli stephensi* were nearly as well neutralized although the latter two venoms were not used in preparing the antivenin and only 5 per cent of *C. v. oreganus* venom was used. Working with South American snake venoms,

Schöttler (1951a) found that the venoms of *Bothrops alternata* and *B. atrox* were better neutralized by four of five heterologous monovalent *Bothrops* antivenins than by their own antivenin. In my experiments, no significant protection was conferred against *Crotalus atrox* or *C. adamanteus* venoms, although both had been used in producing the antivenin. Andrews and Pollard (1953) report, however, that this antivenin confers protection against 16 LD_{50} of *Crotalus atrox* venom per ml. of antivenin and approximately 39 LD_{50} of *C. adamanteus* venom per milliliter of antivenin when injected intravenously into 18-gram mice. The failure to obtain protection against *Agkistrodon* venoms with the antivenin tested is scarcely surprising, since Githens and Wolff (1939) found polyvalent *Crotalus* antivenin had little ability to neutralize *Agkistrodon* venom, and Schöttler (1951b) found polyvalent *Bothrops* antivenin was similarly ineffective.

It made little difference if the antivenin was injected 15 minutes before or 15 minutes after the injection of venom. Slightly better results seemed to be obtained with the latter procedure. Injection of the antivenin into the venom damaged area was somewhat less effective than intraperitoneal injection. This suggests that some of the antivenin may become pooled or nonspecifically bound in the damaged tissues and not be available to neutralize the lethal components of the venom. Animals in this group which survived showed at least as much local necrosis and sloughing as animals in which the antivenin was given intraperitoneally.

It is generally accepted that the antivenins are not equal in potency and specificity to sera produced against bacterial exotoxins. Schöttler (1951a), interpolating from data obtained in experiments similar to those reported here, concluded that 10 to 70 ml. of polyvalent antibothropic serum would neutralize the venom injected by average specimens of Brazilian snakes of the genus *Bothrops*, although 210 to 660 ml. would theoretically be needed in case of bites by very large specimens of *B. jararacussu*, *B. alternata*, and *B. atrox*. Jackson (1944) recommends that 5 to 10 ampules (75–150 ml) of North American polyvalent antivenin be administered in severe cases of snakebite seen in the United States. Shannon (1953) feels that the dangers inherent in the injection of such large amounts of horse serum largely outweigh any possible benefits. The experimental method used here is a stringent one but designed to simulate conditions under which antivenin is actually used. The data give reason to believe that North American polyvalent antivenin has a place in the treatment of snakebite, especially in bites by rattlesnakes of the *Crotalus viridis* complex. If given early and in large doses with proper precautions, it may be a decisive factor in the successful treatment of a severe case. Its failure to show a satisfactory neutralization of *Crotalus atrox* and *Crotalus adamanteus* venoms is a serious deficiency, since these two species account for most of the fatal snakebites seen in the United States. An attempt to improve the neutralizing titer against *Agkistrodon* venoms is also to be desired.

SUMMARY

The protective effect of North American polyvalent antivenin was tested against the venoms of eight representative species of North American pit vipers.

Mice injected with 2 LD_{50} of venom were treated by intraperitoneal or subcutaneous injection of 0.1 ml. of antivenin. Significant protection was obtained against the venoms of the San Diego rattlesnake (*Crotalus viridis helleri*) and the horned rattlesnake (*Crotalus cerastes laterorepens*). Questionable protection was obtained against the venoms of the timber rattlesnake (*Crotalus h. horridus*) and the massasauga (*Sistrurus c. catenatus*). No protection was observed against the venoms of the eastern diamondback rattlesnake (*Crotalus adamanteus*), the western diamondback rattlesnake (*Crotalus atrox*), the copperhead (*Agkistrodon contortrix mokeson*), and the water moccasin (*Agkistrodon piscivorus* spp.). Intraperitoneal injection of antivenin gave slightly better results than local infiltration of the injured tissue. Antivenin does not prevent local tissue necrosis under these circumstances. Neutralization of the venoms observed here is paraspecific; none of the venoms neutralized are used in the preparation of the antivenin.

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ANTIHISTAMINE, ACTH, CORTISONE, HYDROCORTISONE AND ANESTHETICS IN SNAKE BITE[1]

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The not infrequent shortcomings of specific antivenin therapy in snake bite have been exposed in previous papers (for instance Schöttler, 1952b). Such imperfections seem to be due less to limitations of the immunological principle upon which the use of antisera is based, than to a general lack of interest on the part of the manufacturers to improve the often rather questionable quality of the available antivenin preparations. Under these circumstances, reports on allegedly successful treatment of snake bites with the exclusive application of antihistamine (Maier, 1951), adrenocorticotropic hormone (Cluxton, 1951; Hoback and Green, 1953), cortisone (Hoback and Green, 1953), and procaine (Kirsch, 1952) are of special interest. Unfortunately, however, a critical survey of these papers reveals that nothing but the personal impressions of their authors support the favorable opinion on the benefits of such medication in the eight snake bite cases (5 in man and 3 in dogs) which they describe. As a matter of fact, it is virtually impossible to settle the problem in question by clinical observation, because there is no reliable standard by which the severity of naturally occurring snake bite accidents can be judged with any claim of accuracy and, moreover, because the number of cases is far too small to permit statistically justified conclusions as to the merits of any therapeutic measure. Therefore, the only means of determining whether the above-mentioned drugs are apt to replace antivenin, or may serve as an adjuvant of specific therapy, is by accurately controlled animal experiments, as attempted in the present investigation.

MATERIAL AND METHODS

Two different types of lyophilized snake venom were used in the experimental poisoning, namely the chiefly neurotoxic venom of *Crotalus terrificus* (sample L-9), which is devoid of any appreciable local activity, and that of *Bothrops jararaca* (sample L-14), which exerts a pronounced depressor action and causes severe local damage. A crude batch (sample V) of the latter species of venom was employed in the experiments on guinea pigs. The antihistamine assayed was 10-(2-dimethylaminoisopropyl)-phenothiazine (Phenergan),[2] which is considered one of the most potent antihistaminic drugs by Halpern and Ducrot (1946). This preparation was available in the form of its hydrochloride in stabilized solution (Phenergan 117) as well as in the solid state (Phenergan I and II). The adrenocorticotropic hormone employed was the lyophilized commercial

[1] Carried out under Government of the State of São Paulo (Public Health) Contracts reg. nrs. TC 3861-50 and TC 47-53.

[2] Obtained from the Companhia Rhodia Brasileira, Santo André, S. P., through the courtesy of Dr. P. Silva.

TABLE 1

Statistical analysis of the mouse tests with 10 animals per dose

No.	Type of Experiment		Animals				Log-Dose Interval	LD_{50}		χ^2 (degrees of freedom		Heterogeneity Factor	Regression Coefficient		
			Weight gm.		Number										
	Venom	Treatment	Average	Range	Total	Included in statistical analysis		mg/kg	Fiducial limits at $p = 0.05$	Normal	Pooled		b	Fiducial limits at $p = 0.05$	g-criterion
1	saline	Phenergan 117	21.2	18–24	60	60	0.1	251.54	216.07–287.99	4.6(4)			7.09	4.08–10.11	0.18
2		Phenergan 117	18.7	16–23	100	40	0.1	280.23	254.44–308.46	0.3(2)			16.72	7.70–25.73	0.29
3		Phenergan II	18.3	16–21	100	40	0.1	350.29	318.05–385.59	0.4(2)			16.71	7.70–25.72	0.29
4		Phenergan I	18.0	16–22	100	40	0.2	311.33	277.33–358.29	5.3(3)			9.86	5.01–14.71	0.24
5		Phenergan I	18.6	16–25	70	40	0.2	302.92	244.76–373.81	1.5(2)			6.71	3.36–10.06	0.25
6	B. jararaca	saline	19.0	17–23	90	70	0.2	22.57	17.33– 29.50	3.5(5)			3.80	2.34– 5.27	0.15
7		Phenergan 0.1 mg/kg	19.1	18–21	90	70	0.2	19.90	6.15– 63.95	11.4(5)		2.23	2.27	0.85– 3.69	0.67
8		Phenergan 1.0 mg/kg	18.7	17–22	90	80	0.2	13.99	9.73– 19.80	8.1(6)			2.29	1.44– 3.14	0.14
9		saline	18.0	18	70	70	0.2	15.79	9.90– 25.32	1.9(5)			2.08	1.16– 2.99	0.20
10		Phenergan 10.0 mg/kg	18.0	17–21	70	70	0.2	7.05	0.63– 32.80	12.8(5)	10.2(3)	2.56	3.38	1.10– 6.66	0.78
11		Phenergan 20.0 mg/kg	18.5	15–22	70	50	0.2	5.86	4.64– 7.31	3.6(3)			5.80	3.02– 8.59	0.23
12		Phenergan 50.0 mg/kg	18.5	16–24	70	50	0.2	4.79	3.52– 6.06	6.0(3)			5.00	2.37– 7.62	0.28
13	C. terrificus	saline	18.4	17–23	90	70	0.1	0.72	0.62– 0.86	5.1(5)			5.91	3.61– 8.20	0.15
14		Phenergan 1.0 mg/kg	18.0	16–21	90	70	0.1	0.64	0.55– 0.75	6.4(5)			5.89	3.64– 8.13	0.15

15		Phenergan 20.0 mg/kg	18.1	17–22	90	70	0.1	0.77	0.65– 0.91	10.0(5)			5.26	3.18– 7.34	0.16
16		saline	19.2	17–23	90	70	0.1	0.88	0.75– 1.03	3.2(5)			5.83	3.57– 8.09	0.15
17		Phenergan 50.0 mg/kg	19.7	16–22	90	80	0.1	0.65	0.08– 1.00	17.4(6)	9.9(3)	2.91	5.07	1.35– 8.79	0.84
18	B. jararaca	saline	17.6	16–21	80	80	0.2	19.28	13.58– 27.24	8.3(6)			2.35	1.49– 3.21	0.13
19		ACTH 2.5 mg/kg	17.6	16–21	80	80	0.2	14.89	10.77– 20.17	5.6(6)			2.79	1.78– 3.80	0.13
20		ACTH 25.0 mg/kg	17.6	16–21	80	70	0.2	16.57	11.91– 23.21	3.0(5)			2.63	1.60– 3.67	0.15
21	C. terrificus	saline	19.1	15–25	80	50	0.1	0.55	0.48– 0.622	3.7(3)			9.42	4.96–13.89	0.22
22		ACTH 2.5 mg/kg	19.1	15–25	80	70	0.1	0.67	0.58– 0.77	5.0(5)			6.78	4.21– 9.36	0.14
23		ACTH 25.0 mg/kg	19.1	15–25	80	60	0.1	0.71	0.617– 0.81	2.3(4)			7.73	4.58–10.88	0.17
24	B. jararaca	saline	18.1	15–24	80	50	0.2	8.59	6.13– 11.54	1.9(3)			3.42	1.76– 5.07	0.23
25		Cortisone 2.5 mg/kg	18.1	15–24	80	80	0.2	15.73	9.15– 21.19	2.7(6)			1.63	0.88– 2.38	0.21
26		Cortisone 25.0 mg/kg	18.0	15–23	80	60	0.2	13.95	8.91– 18.94	2.3(4)			2.78	1.54– 4.02	0.20
27		Hydrocortisone 2.0 mg/kg	18.0	15–23	80	80	0.2	13.12	9.05– 18.18	5.4(6)			2.46	1.54– 3.39	0.14
28		Procaine 50.0 mg/kg	18.2	15–24	80	60	0.2	6.50	3.43– 9.27	4.1(4)			2.45	1.17– 3.73	0.27
29	C. terrificus	saline	19.5	15–25	80	50	0.1	0.85	0.76– 0.96	2.5(3)			9.89	5.42–14.36	0.20
30		Cortisone 2.5 mg/kg	19.5	15–25	80	60	0.1	0.88	0.77– 1.01	2.1(4)			7.38	4.36–10.40	0.17
31		Cortisone 25.0 mg/kg	19.5	15–25	80	70	0.1	0.98	0.84– 1.13	2.8(5)			6.28	3.90– 8.67	0.14
32		Hydrocortisone 2.0 mg/kg	19.6	15–25	80	60	0.1	1.00	0.88– 1.14	1.5(4)			7.93	4.63–11.23	0.17
33		Procaine 50.0 mg/kg	19.5	15–25	80	70	0.1	0.74	0.63– 0.85	9.1(5)			6.83	3.93– 9.72	0.18

Experiments encased by horizontal lines were carried out simultaneously.

brand ACTHAR.[3] The cortisone was a commercial preparation[4] in the form of a suspension in saline of 11-dehydro-17-hydroxycorticosterone-21-acetate; also the free alcohol 17-hydroxycorticosterone (hydrocortisone)[5] was tested. The procaine solutions were prepared from a commercial brand of p-aminobenzoyl-diethylaminoethanol hydrochloride. In the few tests with histamine, the dichloride[6] was used, but the numerical results obtained with this substance are given in terms of histamine base.

The experiments were carried out on female white mice, injecting the venom subcutaneously, dissolved in a volume of 0.01 ml. per gm. of body weight of physiological saline in the left hind quarter of the animal and, immediately thereafter, the supposed antidote (or saline in the controls) in an equal volume on the opposite side. The locally reacting pharmaceuticals, i.e., hydrocortisone and procaine, were mixed with the venom solution just prior to subcutaneous injection. The effect of the antihistaminic was also examined in female guinea pigs, as mice are practically refractory to the action of histamine. In the guinea pig, venom or histamine, in a volume of 1.0 ml./kg. was injected under the skin above one scapula, and antihistamine above the other. Observation time was recorded till death or recovery. Control tests with venom alone were always run simultaneously with the therapeutic experiments, because the animal response to snake venoms may be subject to considerable variations from day to day, as shown in another paper (Schöttler, 1954). The experimental observations were statistically analyzed after the probit method in the manner described elsewhere (Schöttler, 1954).

RESULTS

As no reliable information on the toxicity of Phenergan was available, this had to be assayed in order to make sure that the doses employed in the therapeutic tests were certainly innocuous for mice. The results of this investigation are recorded in Table 1. The lowest dose of Phenergan 117 capable of killing mice under the conditions of the experiment was a little more than 200 mg./kg.; only this preparation was used in the further experiments. It must be mentioned that many animals which had received considerably lower doses passed days nearly motionless in a deep stupor before they recovered. The highest therapeutic dose of ACTH or cortisone administered in the present study was 25 international units per kg. of body weight. The injection of this dose in mice weighing 15 to 21 gm. was tolerated without any symptoms. The same happened with injections of 25 mg./kg. of hydrocortisone or of 0.1 gm./kg. of procaine. However, lower amounts of the latter two drugs were given in the therapeutic experiments, because observations in a pilot test suggested that these dosages tend to enhance the toxicity of venoms.

[3] Generously supplied by The Armour Laboratories, Chicago, Ill., through the courtesy of Dr. R. J. Meyer.

[4] Cortone (Merck & Co., Inc. Rahway, N. J.)

[5] Furnished by Merck (Pan America) Inc., New York, N. Y., through the courtesy of Dr. A. T. Knoppers.

[6] Kindly donated by Laboratório Torres, Inc., São Paulo, S. P.

TABLE 2

Statistical analysis of the guinea pig tests

No.	Type of Experiment	Animals				LD_{50}		χ^2 (degrees of freedom)	Regression Coefficient		
		Weight gm.		Number							
		Average	Range	Total	Included in statistical analysis	mg/kg	Fiducial limits at p = 0.05		b	Fiducial limits at p = 0.05	g-criterion
34	histamine	492	255–802	20	7	2.24		0.0 (1)	9.21	−5.48–+23.90	2.54
35	histamine + Phenergan	543	260–800	16	14	16.79		11.1 (6)	1.37	−0.59–+3.32	2.04
36	B. jararaca	493	364–599	13	9	4.46		0.0 (1)	9.30	−4.88–+23.49	2.32
37	B. jararaca + Phenergan	492	380–579	13	11	5.71	1.22–80.11	0.3 (2)	4.41	0.15–8.67	0.93
38	C. terrificus	546	381–722	22	15	0.03	0.01–0.05	0.8 (3)	5.40	0.76–10.04	0.74
39	C. terrificus + Phenergan	536	381–645	22	12	0.03		2.0 (2)	8.70	−0.12–+17.52	1.03

The dose of Phenergan administered in experiments no. 35, 37, 39 was 50 mg./kg.

A survey of the various experiments and their results is given in Table 1. It is evident from these figures that the outcome of rattlesnake poisoning was not influenced by any of the drugs employed in this investigation, favorably or unfavorably. The same was true of the *jararaca* venom with the exception that here higher doses of the antihistaminic exacerbated the state of the intoxicated mice so that many of these animals died which would have survived without this treatment (experiments Nos. 11 and 12). None of these forms of medication diminished in a macroscopically discernible degree the local reactions of the *Bothrops* venom, which consist in hemorrhage, gangrene, and subsequent necrosis at the site of injection.

The statistical evaluation of the guinea pig tests (Table 2) suffered from the small number of these animals available in this country. In the majority of the cases, the fiducial limits of the LD_{50} could not be determined owing to an imperfection of the probit method with regard to small numbers, which is discussed in another paper (Schöttler, 1954). Nevertheless, the figures of the mean lethal doses prove beyond doubt that antihistamine does not have the least beneficial effect in snake poisoning in contrast to the appreciable curative action of this drug against fatal doses of histamine.

DISCUSSION

The tentative application of antihistamine in snake bite has a certain justification at least on theoretical grounds. Feldberg and Kellaway (1938) and Dutta and Narayanan (1952) have shown that cobra and rattlesnake venoms liberate histamine from various animal organs on perfusion *in vitro*. Feldberg

and Kellaway (1937) even expressed the opinion that most of the physiological effects of cobra venom could be explained by the release of histamine. The histamine theory has been contested by Dragstedt *et al.* (1939), who found that the amount of histamine liberated by venom *in vivo* is inadequate to account for the degree of the vascular reaction produced. This statement is open to the criticism that the liberated histamine may unite with its target organs so rapidly that the amounts of this substance detected in circulation represent only an insignificant part of the whole quantity set free. Schöttler (1954) opposed the histamine concept, arguing that the guinea pig is extremely sensitive to this drug and the mouse almost completely histamine resistant whereas both animal species are equally affected by venom, which is not compatible with the assumption of a major role of histamine in snake poisoning. However, the observations made in the present study settle the question, at least for the venoms here involved. There can be no doubt that the data of experiments Nos. 34 and 35 supply evidence of the therapeutic efficacy of antihistamine in acute fatal histamine intoxication. It should be emphasized that only the inhibitory effect of antihistamine given prior to histamine has been demonstrated. The simple fact that the antihistamine therapy substantially increased the LD_{50} of histamine for guinea pigs, whereas it left the mean lethal dose of venom unaffected, excludes the histamine theory from further consideration for the explanation of the still unknown physiological mechanism of death in snake poisoning. This does not mean that the histamine released by venoms has no effect at all. The experiments reported by Seba (1949), in which he demonstrated a certain inhibition, by previously given antihistamine, of the local symptoms produced by *Bothrops atrox* venom in man suggest a relation between histamine and these lesions. However, it is rather doubtful if the contribution of this substance to the development of local hemorrhage, gangrene and necrosis is more than very subsidiary, because it is improbable that histamine alone can be responsible for such extensive local damage as that wrought by snake venom. In the present experiments on guinea pigs, fatal amounts of histamine failed to provoke appreciable local reactions, whereas the severe subcutaneous hemorrhages induced by sublethal doses of *Bothrops* venom extended from the backs of the animals down to their bellies.

Though antihistamine has no beneficial effect on the course of snake bite accidents, it may produce a subjective improvement in agitated patients or in painful cases. This is due to the well-known somnolence most types of this drug frequently provoke, and to their local anesthetic potencies, which are equal or even superior to those of procaine as reported by Graham (1947) and Landau *et al.* (1951).

While lower doses of the antihistaminic are without effect in the treatment of snake bite, higher doses of this drug, though in themselves inoffensive, proved to be detrimental in experimental poisoning with the hypotensory *Bothrops* venom. This finding is not at all surprising, as all of the antihistaminic compounds have a strong depressor action according to Glanzmann and Miquel (1949) and others, which on intravenous injection and depending on the dose

will induce an immediate fall of arterial blood pressure with recovery and consecutive development of secondary circulatory collapse. In fact, Yonkman *et al.* (1947) and Graham (1947) even observed that the dose of antihistamine capable of reducing the fall in blood pressure elicited by a certain amount of histamine has the same, or still a greater, hypotensory effect as the histamine, the action of which it is to counteract. Hence, a venom-intoxicated organism which just succeeds in maintaining its blood pressure in a labile equilibrium at the lowest tolerable level, will collapse on the slightest interference, such as provoked by the depressor action of antihistamine. This property makes the antihistaminics a rather ambiguous weapon even in acute histamine poisoning where a careful dosage might be lifesaving, but definitely prohibits their use in snake bite treatment. Therefore, the recommendation of such medication by Seba (1949) and Silva (1952) is not justified.

A theoretical reason for the trial of ACTH and adrenal cortical hormones in snake bite is provided by the concept of the influence of stress on the secretion of these hormones and on the interrelation between them. Severe snake poisoning unquestionably exerts stress on the afflicted organism, but there are scarcely any reports on the effect of venoms on the above-mentioned hormonal system. The hemorrhagic lesions on the adrenal cortex observed by Boquet and Delaunay (1942) after experimental snake poisoning can hardly be considered as typical sequelae of stress because they were also found in kidney, liver, spleen, lung and mesentery. A better, though indirect evidence of the effect of venom on the pituitary gland might be the appreciable hypoglycemia following snake poisoning in the case reported by Staley (1929). Also the four cortisone or ACTH treated cases mentioned above supply no proof for the hormone concept in snake poisoning. The concomitant administration of antivenin within about one hour or less in three of the accidents confounds the issue. Furthermore, the snake species responsible in all four cases was the copperhead (*Agkistrodon mokasen*). Hutchison (1929) reported no fatalities in a group of 171 victims of copperhead bites, some of whom were probably younger than two years, though part of them had not received antivenin. Amaral (1927) mentioned the fatal case of a 14-year-old boy in which medical care and antivenin treatment were applied very late, whereas a 3-year-old boy with serum treatment after 4½ hours recovered and two 6 and 10-year-old boys survived without antivenin application. Thus, it is evident that the copperhead is not particularly dangerous for man and the survival of a patient or an improvement in his condition in such accidents should not necessarily be attributed to any medication. As the hormone theory in snake poisoning is substantiated neither by clinical nor experimental evidence, its further discussion on theoretical grounds and as a part of the shock problem in general, interesting as it may be, would lead too far away from the subject of the present paper.

Though no way of interpretation of the numerical results obtained with ACTH (Table 1) permits the admission of an objective influence of this drug on snake poisoning, it is possible that snake bite patients may feel an improvement of their condition due to the euphoric state the hormone may produce. The

situation is slightly different with respect to the effects of cortisone and hydrocortisone on experimental *Bothrops* poisoning. Here (experiments Nos. 25–27) the mean lethal doses are notably higher than that of the control (experiment no. 24), although the differences are not statistically significant. It might be argued that the considerable error of these determinations, ±35 per cent on the average, could possibly be reduced under more favorable experimental conditions, and would then demonstrate a statistically significant protection of the animals by the adrenocortical hormones. But even in such a hypothetical case the beneficial effect of these drugs would be markedly inferior to that of specific antivenin therapy, which altered the mean lethal doses of the controls from 16.7 to 83.8 mg./kg. (*B. jararaca*) and from 0.67 to 6.35 mg./kg. (*C. terrificus*) in experiments similar to those of the present investigation (Schöttler, 1952a).

In the one reported clinical case where procaine was tried, the patient had been bitten by a common European adder (*Vipera berus*), a species which only rarely produces fatal accidents. Spontaneous recovery within 24 hours is the rule, unless the case is complicated by well-meant but contraindicated measures. The considerations leading to the administration of a local anesthetic in snake bite advanced by Kirsch (1952) and also advocated by Alander (1953), were inspired by a misinterpretation of the rather questionable "neuropathology" of Speransky (1950). In the course of his speculations, the former author is of the opinion that there is no direct connection between venom and the development of hemorrhages at the site of its inoculation, and even that it makes no difference whether snake venom is injected under the skin or directly into a vein. All the snake venoms hitherto examined are considerably more toxic on intravenous than on subcutaneous application, therefore, the theoretical arguments of the above-mentioned authors cannot be taken seriously. The experiments of the present study indicate that procaine, or the more potent antihistaminics, do not influence the course of fatal snake poisoning.

SUMMARY

The therapeutic possibilities of Phenergan, ACTH, cortisone, hydrocortisone and procaine in snake bite were investigated by subcutaneous application of these drugs in mice and guinea pigs previously or simultaneously injected with venom of *Bothrops jararaca* or *Crotalus terrificus* by the same route. The statistical analysis of the results supplied no evidence of a beneficial effect of any of these substances. Higher sublethal doses of the antihistaminic enhanced the toxicity of *jararaca* venom for mice. None of the tested drugs is apt to replace antivenin in snake bite treatment and the use of antihistamine is even strongly contraindicated. The significance of these findings is discussed.

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STUDIES ON COCKROACHES IN A MUNICIPAL SEWERAGE SYSTEM

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The desire to enhance community welfare by preventing contamination of drinking water and food with human dejecta has sound medical as well as aesthetic motivation. The maxim that a properly functioning sewerage system is a necessary prerequisite to the maintenance of a safe and potable water supply has been justified repeatedly by the universal parallel between the removal of raw sewage from water supplies and the elimination or abatement of such epidemic diseases as cholera, typhoid, and paratyphoid. In addition to bacterial infectious agents known to be eliminated via the intestinal tract, in recent years strains of Coxsackie and poliomyelitis virus have been isolated repeatedly from human stools and sewage.

The efficiency of the closed sewerage systems in limiting the spread of gastroenteric entities may be lessened, however, by cockroaches which breed in the manholes and sewer lines. Both flies and cockroaches are outstanding among the omnivorous insects which are of significance in the contamination of food and water as a result of the intimacy and constancy of their association with both the food and the excreta of man and animals. The elimination of privies and the connection of homes to sewer lines deny flies access to human excretory materials; certain species of cockroaches thrive, however, in the sanitary sewers.

General observations and specific surveys made during Texas state-wide vector control activities have indicated that city sanitary sewerage systems are important breeding foci of the American cockroach (*Periplaneta americana*). Of the 67 manholes examined in Austin in September, 1951, 15 had no discernible infestations; in 17 manholes, from 1 to 100 roaches were counted; and 35 manholes were swarming with over 100 roaches. Roaches were visually evident in over 77 per cent of the manholes examined. In Galveston, Houston and Corpus Christi, medium to heavy infestations of the American roach were present in over 50 per cent of the manholes inspected. Comparable conditions have been noted in other Texas cities.

Concern has developed over the possibility that the roaches feeding on human and industrial wastes might be leaving the sewers and entering homes and food establishments. Therefore, a project has been instituted at Tyler, Texas, by the Communicable Disease Center, Public Health Service, Atlanta, Georgia, and the Texas State Department of Health to study the bionomics of the sewer cockroaches. Two entomologists have been assigned to Tyler to perform the field duties of the project. Engineering, entomological and medical supervision

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has been supplied by the Tyler-Smith County Health Unit, Texas State Department of Health, and by Communicable Disease Center personnel, both in Atlanta, Georgia, and assigned to the Texas State Department of Health, Austin. This report covers project activities from August, 1952, through September, 1953.

Principal activities may be categorized as follows:

1. Routine, visual inspection of 80–90 manholes weekly in order to determine possible seasonal or other shifts in population densities.
2. Collection of large numbers of sewer roaches for marking and return to the sewers, with extensive efforts to recover the marked roaches in adjacent homes in order to map their peregrinations.
3. Trials with different types of plumbing traps to determine what obstacles they offer to the progress of roaches into homes from the sewer lines.
4. Collection of roaches from the sewers for pathogen isolation studies.

POPULATION DENSITIES

In August, 1952, an initial manhole survey was made in Tyler, Texas, to ascertain the per cent and degree of roach infestation. A total of 670 manholes was examined during this survey, although subsequently the number of manholes included in the study period was raised to 762. An arbitrary standard of 1 to 25 roaches was selected as a light infestation, 25 to 100 as medium, and 100-plus as a heavy infestation. Of the 670 manholes examined originally, 403 (60.1%) had no infestation, while 115 (17.2%) were light, 64 (9.6%) were medium, and 88 (13.1%) were heavy.

No correlation was noted between the abundance of roaches in the sewers and the socioeconomic level of the adjacent homes or business establishments. Similarly, these insects were not more closely associated with any ethnic group. For convenience in making regular manhole inspections to determine seasonal

TABLE 1

Pathogens isolated from Periplaneta americana *collected from Tyler, Texas, sewer manholes*

Number of Roaches		Date Collected	Manhole Number	Pathogens
Nymphs	Adults			
3	2	9–23–52	103	*S. oranienburg*
1	4	9–29–52	239	*S. meleagridis*
5	0	9–29–52	193	*S. tennessee* & *S. rubislaw*
2	3	10–27–52	193	*S. panama*
4	1	9–29–52	216	*S. newport*
0	5	10–7–52	216	*S. newport*
3	2	9–30–52	247	*S. tennessee*
3	2	10–13–52	239	*S. meleagridis*
5	0	11–17–52	239	*S. kentucky*
5	0	10–13–52	353	*S. bareilly*
0	2	10–28–52	436	*S. oranienburg*
1	4	11–18–52	679	*S. anatum*

or other shifts in population density, the city has been divided into 8 sections of approximately equal size. The sections have been subdivided into 73 stations of about 10 manholes each. Weekly visual inspections have included 10 manholes in 2 stations (selected at random) from each of the 8 sections, with the exception that 4 stations were examined in one of the sections for most of the study period. Thus 80–90 manholes have been surveyed weekly. Of these 5 manholes from each station, 3 are fixed and 2 are random.

During these routine density studies, an attempt has been made actually to count the roaches in the manholes, rather than to place each manhole in one of the 4 categories (no infestation, light, medium, or heavy infestation) utilized in the initial survey. Admittedly, this visual method of sewer roach population density determination has its limitations. Only the roaches remaining, at least momentarily, in the manhole when the cover is raised are available for counting; nothing is known of the number in the sewer lines. Also, it is more difficult to count the roaches in manholes 12 or 15 feet deep than in those 4 or 5 feet in depth.

Monthly averages of the results of the manhole population counts are given in Figure 1. It is evident that the roaches are abundant throughout the year, with the smallest numbers being encountered in February and March. An increase was noted from April through September. The fact that the peak month was September does not necessarily mean that there were more roaches in the

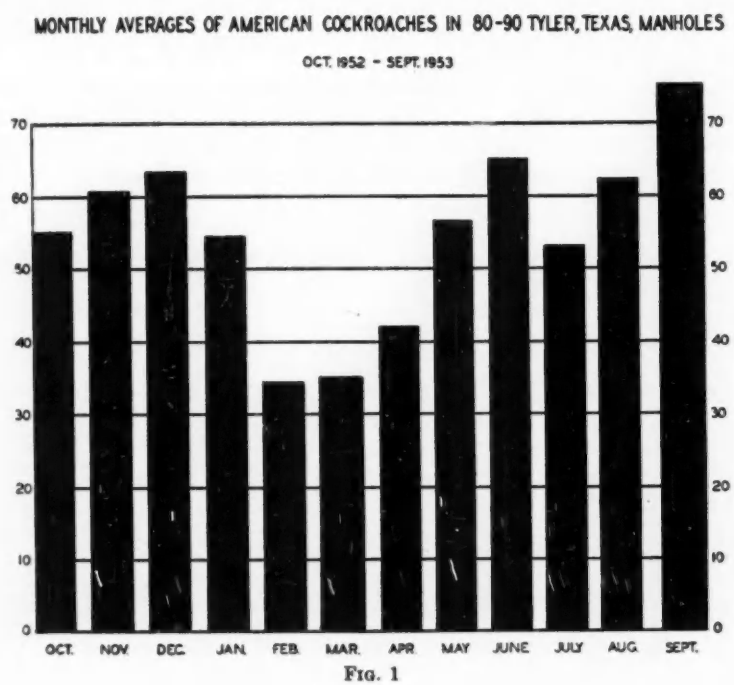

FIG. 1

sewers at that time, but simply that there were more in the manholes to be counted. A plausible explanation for the population fluctuation is that changes in sewer line temperature, even though slight, stimulate the insects into increased activity, causing larger numbers to move from the lines into the manholes.

Thermograph and hydro-thermograph readings have shown that the sewers and manholes provide optimum developmental conditions for American roaches, and this helps to explain why the sewerage systems in our municipalities are such important breeding foci of these insects.

During the study period (August, 1952, through September, 1953) the temperature and humidity in the manholes have been checked regularly. From February 2 to 15, 1953, the minimum manhole temperatures averaged 55.3°F. (range 45°–59°) with a variation of only a few degrees between the minimum and maximum temperatures. For the same period of time the minimum atmospheric temperatures averaged 42.4°F. (range 33°–49°).

On July 20, 1953, the manhole temperatures were taken at 8:30 A.M. and hourly until 11:30 P.M. and averaged 77°F. with a variation of only one degree above and below this temperature. Atmospheric temperatures taken the same day and hours averaged 84°F. (range 79°–97°).

SPECIES OF ROACHES IN SEWERS

Hebard (1943) gives a key to 31 species of cockroaches which are listed as "undoubtedly being present in Texas." More recent publications have added a few species to this total, e.g., *Blattella vaga* has been reported from the Rio Grande Valley of Texas by Riherd (1953). The mildness of the climate along the Gulf Coast is especially propitious for the establishment of tropical species introduced by commerce. Of the roaches listed by Hebard (loc. cit.) as occurring in Texas, at least 8 are classed as introduced species and not as indigenous forms.

The potential importance of tropical species to our society is emphasized by the case of the brown banded roach, *Supella supellectilium*. Apparently a native of Africa, the species was first reported in the United States (Florida) in 1903 by Rehn. It was not noted as a pest until 1929, when its occurrence in a private home in Nebraska was reported. The species has spread until it is now one of the principal domiciliary species in the United States.

Five species of cockroaches have been recovered from the Tyler manholes: the American roach, *P. americana*; the smoky brown roach, *Periplaneta fuliginosa*; the oriental roach, *Blatta orientalis*; and the wood roaches, *Parcoblatta bolliana* and *P. pennsylvanica*. Approximately 40 per cent of the 762 manholes concerned in this study were infested with *P. americana*. *B. orientalis* were in 10 manholes, *P. fulginosa* were in 3, *P. bolliana* were in one, and *P. pennsylvanica* were in a single manhole. Mixed infestations of *P. americana* and one of the other roach species were the rule in the 15 manholes in which forms other than American roaches occurred.

Two species which commonly infest premises, the German roach, *B. germanica*, and the brown banded roach, *S. supellectilium*, were not taken from the Tyler sewer manholes.

DISPERSAL STUDIES

The demonstration of the prevalence of cockroaches in sewerage systems raises the important question as to whether or not these insects leave their harborages and enter homes and business establishments in significant numbers. There are 2 obvious avenues of premises encroachment—by egress from the manholes through holes in the cover and then walking or flying to homes where admittance can be effected under doors or screens, or by following the sewer lines into homes and entering via the water traps through sinks, bathtubs, or commodes. Both of these avenues of approach have been extensively investigated.

An abundance of visual evidence that the American roaches breeding in the sewers by no means confined their activities to the sewers, at least during the summer months, was obtained by regular nocturnal manhole observations. As many as 11 roaches were seen to leave a manhole within an hour period. It remained to be proven that these fecally contaminated insects entered homes and business establishments. To this end, in May 1953, 1000 roaches were lightly anesthetized, painted on the thorax with a green lacquer enamel,[3] and returned to the manhole from which they were originally obtained. Several dyes were tried in marking the roaches, but none was very effective due to the oily character of the roach thorax. It was found that sufficient lacquer enamel remained on the roaches to afford recognition for a period of at least 6 weeks.

A news release was issued to the local papers to inform the Tyler citizens of this particular facet of the project. The cooperation of the people living in the immediate neighborhood of the release point was solicited in making available to the City-County Health Unit information as to the presence of painted roaches in their homes. Project personnel also visited adjacent houses, and when permission was granted, utilized freon-pyrethrum bombs in kitchens to aid in the search for marked American roaches.

The first recovery of a marked roach was from an apartment house immediately adjacent to the release point, 6 days after the experiment was initiated. Within a 3-week period 15 painted American roaches were recovered from the same apartment house and so many other study roaches were seen by the residents that they demanded that the manhole be sprayed to eliminate the insects. The apartment was definitely a submarginal dwelling and its torn screens and doors offered few obstacles to entry by roaches. Almost 2 months after the painted roaches were released in the manhole, 2 of the roaches were taken from another manhole approximately 385 yards down the street, and one of the experimental roaches was taken in a grocery store one block from the release site.

A second release of 1000 painted roaches was made in a manhole at the corner of S. Scott and W. First St. May 25, 1953. The first residential recovery of a single roach was made May 29 at a home immediately adjacent to the release manhole. Additional recoveries of painted roaches were made from the same

[3] "Glo-Craft" trademark of Switzer Brothers, Cleveland, Ohio, was used. It is assumed that competitive products of comparable quality would be equally effective.

home. One was collected on June 1, and 3 on June 9. It could not be ascertained whether admittance was gained from outside the house or through the sewer lines.

A third group of 1000 painted cockroaches was released June 15, 1953, in the manhole at the corner of N. Moore and W. Harmony St. Three days later 4 of the roaches were killed in a home in the same block.

A fourth release of 1000 marked roaches was made July 10, 1953, at the corner of S. Niblack and E. Houston St., and a week later a marked roach was recovered from a home directly across the street from the release site.

It is anticipated that approximately 1000 painted roaches will be released in sewer manholes every month for at least 12 months in an effort to determine whether or not the roaches continue to disperse during the cooler months. The studies to date have conclusively demonstrated that these insects leave the sewerage system and enter homes as far as a block away. While only a small percentage of the study roaches have been taken in homes, the recovery techniques were, of necessity, fairly insensitive.

Certain investigations were made with the 2 common types of plumbing traps used in connection with sinks, bathtubs, and commodes to determine the possibility of the cockroaches entering homes from the sewer lines. It has been shown by project personnel that roach dispersal exists from manhole to manhole by means of connecting lines.

Models of a "P"-type trap similar to those in use under sinks and built into commodes and a "drum"-type trap used under bathtubs were obtained from a plumbing contractor. On January 9, 1953, 16 American roaches were placed in a small bobbinet cage attached to one end of a "P"-type trap having the usual water baffle. Another cage was placed over the other end, with banana as an attractant. By January 15, one of the roaches had passed the barrier. A similar experiment was tried on February 9, 1953, and within a few days 3 of 16 roaches had passed through the water. On April 21, 1953, 49 roaches were caged at the lower end of a "drum"-type trap, also using water as a deterrent. In 2 days, 2 roaches had gone through the trap, and by April 28, a total of 4 had passed through.

PATHOGEN ISOLATIONS

Samples composed of 3–5 roaches have been collected from 10 to 15 manholes at regular intervals throughout the year and forwarded alive to the Texas State Department of Health Laboratory for disease organism isolation studies. Each sample has been routinely macerated or ground in a sterile mortar and suspended in physiological saline and viral and bacterial isolations have been attempted. Cultural procedures that were followed in a search for enteric bacteria are those routinely employed in diagnostic laboratories. Virus studies have been limited to the inoculation of infant mice, and only negative results have been obtained.

Results of the *Salmonella* recoveries are given in Table 1. *Salmonella* were isolated from 12 of the 360 pools studied. All of the *Salmonella* were from the 1,650 American roaches, *P. americana*, in 323 pools taken from sewer manholes.

There were no recoveries from the 70 American roaches in 5 pools studied from food establishments; from the 54 oriental roaches, *B. orientalis*, in 15 pools from sewer manholes; the 235 brown banded roaches, *S. supellectilium*, in 11 pools from food establishments; or from the 118 German roaches, *B. germanica*, in 5 pools from food establishments.

SUMMARY

Approximately 40 per cent of the sewer manholes in Tyler, Texas, have been found to be infested with the American roach, *Periplaneta americana*. Five species of roaches were taken from the sewers: *Periplaneta americana*, *P. fuliginosa*, *Blatta orientalis*, *Parcoblatta bolliana* and *P. pennsylvanica*. Nine species of *Salmonella* have been isolated from American roaches collected in the sewer manholes: *S. bareilly*, *S. tennessee*, *S. newport*, *S. panama*, *S. rubislaw*, *S. meleagridis*, *S. oranienburg*, *S. kentucky*, and *S. anatum*. It has been definitely determined that sewer cockroaches leave the manholes and enter homes as far as a block away. Roaches may enter homes via the sewer lines, as it has been demonstrated that under experimental conditions they will pass through the plumbing traps currently used under sinks, bathtubs, and commodes.

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Diseases of the Skin for Practitioners and Students. by George Clinton Andrews, M.D., F.A.C.P. Clinical Professor of Dermatology, College of Physicians and Surgeons, Columbia University; Attending Dermatologist to the Presbyterian Hospital, Columbia-Presbyterian Medical Center, New York. 4th edition: 877 pp., 777 illustrations. Philadelphia and London: W. B. Saunders Co. 1954. Price $13.00.

BOOK REVIEWS

Distribution of Rickettsial Diseases. III. Tick and Mite Vectors, by Dept. of Medical Geography, American Geographical Society, New York: American Geographical Society, 1954. Price $1.25 folded, $1.50 flat.

This is another in the series of excellent maps prepared by the American Geographical Society and showing the distribution of arthropod vectors of disease. The present map shows the general areas of occurrence of the arthropods involved in the transmission of rickettsial diseases. The area of distribution given for the various tick and mite vectors represents the area in which the vectors have been reported as present and not intended to be interpreted as limitative. The vectors dealt with on the map belong to the orders Hemiptera and Acarina. The Acarina dealt with comprise the three families Argasidae, Ixodidae and Trombidiidae. The families Cimicidae (order Hemiptera) are included in the discussion on the reverse side of the map, together with the other vectors mentioned above.

The distribution of the several vectors is very clearly presented, and the reader can obtain at a glance a very good idea of the areas of occurrence of these arthropods. On the map are shown only those vectors which have been definitely proved to be concerned in the transmission of rickettsial diseases. Other arthropods which transmit only occasionally or are found infected in nature but not proved to transmit to man or are experimental vectors, are dealt with in a tabular presentation on the reverse side of the map. This resume of selected species is given in tabular form with the listing of each species, together with its most common hosts, the rickettsial disease transmitted, the importance of the species as a vector, and its geographical distribution.

E. H. LENNETTE

Smallpox Vaccination, a Survey of Recent Legislation. WHO, Palais des Nations, Geneva, 1954. Reprinted from the *International Digest of Health Legislation*, 1954, **5**: 221–262. Price 3/6, $0.50, Sw. fr. 2.

This is one of a number of comparative surveys of health legislation in most of the countries of the world. Smallpox is a constant threat to countries in which it has virtually disappeared, from the endemic regions in Asia, Africa and America, and quarantine is small protection since introduction of the disease through mild cases or those in the incubation period becomes known only when it begins to spread. There are great divergences in the legislation of the fifty countries included in this survey. In 1949 the Pan-American Sanitary Bureau approved a proposal to carry out a program for the eradication of smallpox throughout the Americas. It will come as a surprise to many that the legislation of seven states of the U. S. A. prohibits compulsory vaccination. There is a useful appendix giving a synoptic table of legal requirements in fifty countries regarding vaccination and its technique, and a list of sources of information.

L. W. HACKETT

WHO Expert Committee on Rabies, Second Report. Technical Report Series No. 82. 27 pp., WHO, Geneva, 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price 1/9, $0.25, Sw. fr. 1.-

This report reviews advances in the field of rabies during the past three years. Of particular significance are the sections treating with antirabies hyper-immune serum, chick-embryo-adapted virus vaccine, and modifications in chick-embryo-adapted Flury strain rabies virus in high egg-passage. Hyperimmune serum and 14 subsequent daily doses of phenolized vaccine is recommended for treating persons with severe exposure. Studies upon which this recommendation is based are summarized. A useful table of indications for specific post-exposure treatment using serum is included. Field trials in Israel and Malaya demonstrating the effectiveness of chick-embryo vaccine in dogs are summarized. Chick-embryo vaccines are not yet recommended, however, for human treatment. Rabies control in animals including international transfer of animal, diagnosis, suggested research, potency testing of vaccines and hyperimmune serum, and epizootiology of wild-animal rabies including rabid insectivorous bats in the U. S. A. are other subjects covered.

This will be found a most useful report by all concerned in the control of this zoonosis which is of such importance in so many countries.

GEORGE L. HUMPHREY

Les Animaux de Laboratoire (Anatomie, Particularités Physiologiques, Hématologie, Maladies naturelles, Expérimentation). By JULIEN DUMAS, Flammarion publ., Paris 1953, 720 pp., bound, $12.

This remarkable book should prove indispensable to all investigators—and especially so to microbiologists—whose work involves animal experimentation. We do not know of a more comprehensive treatment of the subject in any language, yet it is presented very concisely, with constant attention to practical problems.

The first section describes the anatomy, physiological peculiarities, hematology and breeding conditions of various laboratory animals (guinea pig, rabbit, mouse, rat, hamster, ferret, spermophile, hen, pigeon, monkey, cat and dog). The second section contains 50 pages of valuable information on the housing and feeding of these animals, as well as on the types of cages and their cleaning and disinfection. The third section describes, in great detail but in a concise and very orderly way, the various bacterial, viral, protozoan and helminthic diseases naturally occurring in the guinea pig, rabbit, mouse, rat, ferret, hen and monkey. The consequences of avitaminosis, the tumors and the ectoparasites are also described. The fourth section describes the techniques of animal experimentation: marking, handling, anesthesia, collection of samples, modes of inoculation, autopsy and sanitary disposal of the corpses.

The book contains 163 drawings, sketches and photographs and a good index. After each

chapter, there is a rather complete list of references, and the bibliography is truly international in scope. The printing and the paper are excellent.

G. H. WERNER

WHO Expert Committee on Malaria, Fifth Report. Technical Report Series No. 80. 42 pp., WHO, Geneva, 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price $.25, 1/9, Sw. fr. 1.

This exceedingly useful report should be in the hands of all who have to deal with malaria in the field or teach it in the schools. It sums up our knowledge and progress to date in the use of insecticides (especially the experience with interrupting application at a certain stage) and in chemotherapeutics of malaria, with the part that newly synthesized antimalarials might play in malaria control. There are valuable appendixes on measurement of longevity of mosquitos; technic for assessing susceptibility of anophelines to insecticides; criteria of malaria eradication; and the mode of action of antimalarial drugs, with a list of supporting documents.

L. W. HACKETT

GUTHE, T. & R. R. WILLCOX, **Treponematoses, A World Problem,** WHO, Geneva, 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. 79 pp. Price 3/6, $0.50, Sw. fr. 2.-

This WHO booklet describes the progress realized since World War II in combatting this group of infections. The effectiveness of penicillin and the organization of campaigns to reach those in need of treatment have had considerable impact on this health problem in areas where syphilis, yaws, and other endemic types of the infection are prevalent and where a "mass" approach has given hope of their eventual control. The book treats of the epidemiologic history of treponematoses, the control methods in use, and the integration of these measures with the general public health services. Besides maps, graphs and tables, the book contains a selective bibliography and will interest not only specialists but also the general public health worker and all concerned with tropical medicine and international health activities.

L. W. HACKETT

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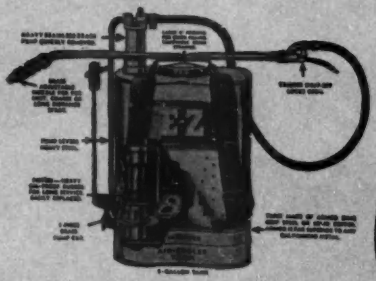

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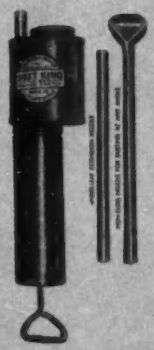

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